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Effect of Infra-Red Radiation on Growth of Rachitic Rat.

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The results of a series of experiments on the biological effect of isolated bands of radiation, show that a 10 minute daily exposure to near infra-red radiation, (ranging from 720-1120$\mu\mu$) of an intensity 0.132 gm. cal/min/cm^2, will produce a definite effect upon young rats fed upon a rickets-producing diet. This effect is not fully apparent until the 5th or 6th week of experiment. The radiation causes increased growth of the animals, and prolongs by a period of 4 to 6 weeks their survival on the rickets-producing diet, without conferring any protection against the disease. Infra-red radiation, therefore, acts in a manner different from ultra-violet, and exerts a physiological action whose effect can be demonstrated.

In a limited number of animals examined, this growth-promoting effect of infra-red radiation was found to be associated with marked enlargement and hypertrophy of the thyroid gland. Irradiation with ultra-violet, in corresponding litter-mates, was found to prevent this hypertrophy whether or not the rat was exposed to infra-red radiation. This result must be corroborated by further experiment.

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* Medical Travelling Fellow of the Rockefeller Foundation, and Senior Sterling Fellow of Yale University.

The expenses of this investigation have been defrayed from a Gift Fund of the Department of Pediatrics, Yale University.

Some hyperplasia of the parathyroid gland was observed in a group of rats receiving infra-red radiation. The glands of the corresponding group, receiving ultra-violet, were of normal size.

Ash analysis of the bones of 18 rats protected against rickets with ultra-violet radiation showed slightly higher ratios between the ash and organic residue, than those of a similar group of litter mates that had received in addition to the ultra-violet, a daily exposure to near infra-red radiation.

These experiments have been in progress, in collaboration with Mr. L. A. Jones of the Eastman Kodak Company, for the past two years.

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Some Experiments on Reducing Reaction of Cerebro-Spinal Fluid.

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Benedict's copper method for determining blood sugar[1] has been applied to the study of a considerable series of spinal fluid specimens. The values obtained agree almost exactly with published results obtained by the technique of Folin and Wu. A series of direct comparisons described by Lyttle and Hearn[2] and confirmed by a few experiments in our own series showed that the difference was approximately 3 mg. %, expressed in terms of glucose. The Folin method gave the slightly higher values. It seems probable that the non-glucose reducing compounds which cause rather marked differences in the sugar values in whole blood and plasma do not enter the spinal fluid in appreciable amounts.

Since the compounds which reduce the "uric acid reagents" in acid solutions, but which are not precipitated by silver lactate are supposed to be in part responsible for these differences in blood, studies were carried out to demonstrate their presence in or absence from the spinal fluid. They either were absent, or were present in traces so slight that they could not be demonstrated.

The rate of reduction of Benedict's copper reagent by pure glucose and by spinal fluid was compared. After heating for 1 minute the apparent sugar was 1½ to 2 times as high as when the heating was continued longer. If the time of heating was between

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[1] Benedict, S. R., *J. Biol. Chem.*, 1925, lxiv, 207.

[2] Lyttle, J. D., and Hearn, J. E., *J. Biol. Chem.*, 1926, lxviii, 751.

3 and 15 minutes the values obtained were constant. Long periods of heating—30 minutes to 1 hour—gave lower values. The reduced copper is therefore oxidized more rapidly in spinal fluid preparations than when reduction is brought about by pure glucose in water. Benedict has stated that similar changes are observed in blood filtrates. The more rapid reduction during short periods of heating may be due to a similar cause, or may be brought about by the presence of a small amount of a very readily oxidized material in the fluid.

Although there seems to be a small amount—about 5 mg. %—of reducing material in the spinal fluid which is not fermentable by yeast, the amount which is not destroyed by the colon bacillus, and which is therefore almost certainly not a carbohydrate, is very small. It seems to be present in concentrations of about 1 mg. % expressed as glucose.

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Action of Pilocarpine on Pupil of Guinea Pig.

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From the Department of Pharmacology, Syracuse University.

It has been shown that pilocarpine dilates the normal and constricts the denervated pupil of the rat. The mode of its action has also been discussed in a previous paper.[1] The normal pupil of the rat is constricted, due to the powerful sphincter tone of the iris. The purpose of this investigation is to determine how a naturally dilated pupil (weak sphincter tone) would react to pilocarpine.

Solutions of pilocarpine hydrochloride (2-10%) were instilled into the conjunctival sac of pigmented and albino guinea pigs. Pilocarpine caused in every instance dilation of the pupil and abolished the light reflex. The result was even more striking if the pupil was first reduced in size by sectioning the cervical sympathetic. The same solutions of pilocarpine invariably produced pupillary constriction in rabbits. The pupil dilated by pilocarpine did not react to usual quantities of arecoline, muscarine and physostigmine (0.5-1% solution), the latter drugs acting orthodoxly in normal guinea pigs. Atropine and "sympathomimetic" drugs increased the size of the pupil dilated by pilocarpine.

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[1] Koppányi, T., *J. Pharm. and Exp. Ther.*, 1928, xxxiv, 73.

The section of the short ciliary nerves reverses the pilocarpine action in guinea pigs, its effect being constriction of the pupil. Atropine abolishes the miotic action of pilocarpine in denervated eyes.

Pilocarpine thus produces pupillary dilatation due to paralysis of parasympathetic myoneural junctions in the normal eyes; and constriction due to stimulation of the same junctions in the denervated eye of the guinea pig.

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Action of Sympathomimetic Drugs on Pupil of Guinea Pig.

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From the Department of Pharmacology, Syracuse University.

Meltzer and Auer[1] showed that subcutaneous injections or instillations of epinephrin have no effect upon the normal pupil of the cat and rabbit. Ephedrine and cocaine, however, produce mydriasis even in normal animals. Ergotoxin, or ergotamine, are said to cause miosis in dogs and cats and mydriasis in rabbits.

In guinea pigs the instillation of a few drops of epinephrin hydrochloride, 1:1000, causes a maximal dilatation of pupils of normal animals and of animals whose cervical sympathetic has been cut below the superior cervical ganglion. Ordinary doses of arecoline and physostigmine do not constrict the pupil dilated by epinephrin. There is a complete loss of light reflex. Ephedrine and cocaine behave exactly like epinephrin.

Ergotamine tartrate administered by instillation into the conjunctival sac produces mydriasis. But injection of the drug into the anterior eye chamber causes, after an initial dilatation, constriction of the pupil, which lasts for at least 12 hours. The pupils constricted by ergotamine do not react to other sympathomimetic drugs, but do respond to atropine.

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[1] Meltzer, S. J., and Auer, C. M., *Am. J. Physiol.*, 1904, xi, 28, 37, 40.

4148

Compensatory Eye Positions in Complete Torticollis in Rabbit.

THEODORE KOPPANYI.

From the Department of Pharmacology, Syracuse University.

An albino rabbit showed drooping of the right ear, then developed torticollis, which progressed until the head and neck became twisted 90° to the vertical. The right eye was directed straight upward. In this head posture the position of both eyes were normal, no vertical deviation being present. But when the head was forcibly brought back into the normal posture, (the axis of the body remaining the same), the right eye rolled upward and the left eye downward. This reversal of the compensatory vertical eye positions is in striking contrast to the observations of Magnus[1] on unilaterally labyrinthectomized rabbits. When head and neck were allowed to assume their original posture, both eyes moved back into their normal positions.

Four weeks later the animal's head recovered its normal posture and both vertical and rotatory compensatory eye positions were found to be normal.

The cause of this remarkable disturbance was a tumor of the middle ear, which destroyed adjacent structures.

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Certain Characteristics of Formation of Conditioned Reflexes in Sheep.

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The defensive reaction evoked by the application of an electric shock to the sheep's foreleg is a convenient unconditioned reflex from which to develop various conditioned reflexes. Six lambs born in May, 1928, were, after about 18 days preliminary training in August, caused to develop auditory conditioned reflexes of the simultaneous type. That is, the auditory stimulus signalling an electric shock preceded the shock by a short, variable interval of 2 to 6 seconds. The conditioned stimulus for 2 of the lambs was the sound of a buzzer. For the others it was the sound of the metronome beating twice per second. The conditioned reflex appeared in the 6 animals after 3 to 7 combinations of sound and shock and became relatively stable (11 or more positive conditioned responses in succession) after 18 to 43 combinations. The development of a conditioned reflex of the type described and a simple maze habit exhibit a common characteristic. Both show a period of instability between the first appearance of the new response and the final, more or less stereotyped, reaction.

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[1] Magnus, *Koerperstellung*, 1924, 166. Berlin.

The most interesting feature of the formation of the conditioned reflex was the concentration of the motor response with increasing training. At first, the reaction involved a widespread activity of the skeletal musculature. The leg receiving the shock might be extended instead of flexed as the animal struggled in the harness. Later, and usually before the conditioned reflex was stable, the response was gradually concentrated in the leg stimulated by the electric current and the sound now evoked a brief and precise leg movement. The reaction of the other skeletal muscles at this stage served to give the body a posture in which the foreleg could be vigorously flexed.

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Note on Tea as a Source of Vitamin C.

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With the exception of cabbage and lettuce the leaves of plants have not been examined quantitatively for their content of vitamin C. The observations of Miura[1] indicated that different kinds of tea possessed widely different amounts of vitamin C, and the quantity found in green tea compared favorably with the content of the usual anti-scorbutic foodstuffs. Fermentation and its accompanying oxidative changes are a part of the commercial processes of tea manufacture and it seemed desirable to confirm Miura's observations with the possibility of correlating the data with the accepted facts relative to the oxidative destruction of vitamin C.

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[1] Miura and Tsujimura, *Proc. Jap. Assoc. Agr. Chem.*, 1924, i, 1; Miura and Okabe, *Publ. Assoc. Tea Merchants*, Feb., 1926.

A series of 300 gm. guinea pigs on Sherman's[2] basal ration (with equal weights of rolled oats and wheat bran) was used for curative tests. These animals gained their last 50 gm. of body weight on the basal ration plus a leaf of cabbage. After the latter was omitted scurvy developed in from 2 to 4 weeks. Due to too long a delay in initiating the curative regime some animals succumbed within a day or two after its beginning, and thus served, along with others by design, as controls. Other controls proved the prolonged adequacy of the basal ration plus cabbage. None of these animals appears in the tables.

Four kinds of tea were tested in the form of infusions and the results are given in the following table. The preparation of the tea infusion was carried out as uniformly as possible. Fresh infusions were made daily; distilled water and glass flasks were used. The infusions made would probably pass for fairly strong tea. Two gm. of tea were used for 100 cc. of boiling water. Directly after the tea was added the flame was turned out and the flask was shaken several times during the succeeding 5 minutes. At the end of that time the liquid was decanted from the leaves which had settled to the bottom. The volume was restored to 100 cc. and in order to secure complete consumption of the desired amounts the tea was slightly sweetened by the addition of 3 gm. of pure cane sugar to 100 cc. of the tea. The amount consumed was carefully observed and any liquid remaining unconsumed was subtracted and due correction was made in the records. Animals on the larger allowances of tea were often deprived of water at night to insure the prompt consumption of the next day's dosage.

The minimum daily curative dose of tea infusion (2%) from different teas appears to be as follows: Japan Green Pan Fired, between 10 and 15 cc. Japan Green Basket Fired, not less than 20 cc. Oolong, not less than 25 cc. Orange Pekoe, considerably more than 25 cc.

Presumably these differences may be related to the method of preparation rather than to differences in plants or in their habitat. In the preparation of green tea the leaves are dried without fermentation. Fermentation previous to drying results in black tea. Sauer kraut and ensilage are reported to be devoid of vitamin C probably for the same reason. The kidney tissue of some of the animals was kindly examined by the Department of Pathology. This Department reported cloudy swelling and did not consider the condition significant.

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[2] Sherman, H. C., LaMer, V. K., and Campbell, H. L., *J. Am. Chem. Soc.*, 1922, xliv, 165.

TABLE I. *Amounts of Tea Infusions Necessary to Prevent Scurvy.*

Animal No.	Amt. Infusion Daily cc.	Days Fore-period	Gm. loss During Fore-period	Days of Curative Period	Gm. Change During Curative Period	Autopsy Findings.†
31	10[1]	24	8	50	+18	Discontinued. No scurvy. Complete cure.
29	10	19	37	34	+28	Died of pneumonia. No scurvy.
15	15	24	88	5	—46	Severe scurvy. Fore-period perhaps too long.
10	15	32	61	30	—59	Discontinued. No scurvy. Kidney degen.
7	15	25	38	59	— 5	Discontinued. Very mild scurvy.
12	20	21	81	52	+ 8*	No scurvy. Died of kidney disease.
22	10[2]	25	76	5	—36	Scurvy. Complicated with pneumonia.
19	10	23	31	4	—63	Acute scurvy. Necrosis of liver. Fore-period probably too long.
2	10	27	76	44	—75	Scurvy. Kidneys congested.
30	15	25	53	71	+63	Mild scurvy. Kidney degeneration.
27	20	28	2	43	+30	Mild scurvy.
4	20	25	74	18	—70	No scurvy. Lung infection.
43	15[3]	21	62	9	—48	Mild scurvy. Kidney degeneration.
37	15	25	81	18	—46	Severe scurvy.
36	20	48	5	11	—56	Severe scurvy but compl. with lung infec. Fore period perhaps too long.
35	20	29	30	18	—54	Mild scurvy. Kidney degeneration.
40	20	22	48	21	—37	Scurvy but joints not large.
38	20	19	51	28	—71	Marked scurvy.
41	25	25	54	26	— 8	No scurvy. Kidney degeneration.
11	10[4]	26	62	24	—36	Scurvy complicated by lung infection.
28	10	28	65	51	—68	Extreme scurvy.
24	15	30	+25	22	—112	Marked scurvy.
16	15	19	38	16	—90	Mild scurvy. Pronounced lung infection.
32	20	40	+22	43	—21	Anesthetized. Mild scurvy. Kidney degen.
18	20	22	99	7	—75	Severe scurvy.
21	20	30	25	12	—22	Scurvy.
3	25	19	68	7	—16	Severe scurvy.
25	25	25	53	16	—57	Severe scurvy.

*Gained 95 gm. during curative period but declined rapidly at end.

†With a few exceptions, as noted, all the animals died of scurvy unless other cause is indicated. The others were anesthetized for examination.

[1] Japan Green Pan Fired. [2] Japan Green Basket Fired. [3] Oolong. [4] Orange Pekoe.

In every-day nutrition, despite its fairly high content of vitamin C, green tea has limited value as an anti-scorbutic. It can not be used by children and should not be used by adults to replace natural food-stuffs which meet additional nutritive requirements. Tea is a never-failing item in the rations prepared for expeditions, military or otherwise, into infertile or difficultly accessible regions. It is not known how long the vitamin C of tea survives the condition of storage and shipment. Presumably vacuum packing might be advantageous.

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Cause of Hen-Feathering in Campine and Bantam Males.

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In ordinary breeds of the fowl differences in secondary sexual characteristics of the plumage have been shown[1] to be due to endocrine factors which are largely controllable under experimental conditions. With the type of feather produced serving as a measure of response, evidence from various sources is consistent in indicating that in most breeds the follicles of the male and those of the female react in exactly the same manner to any set of stimuli.[2] In other words, sexual differences in plumage are referable to differences in endocrine stimuli rather than to differences in tissue response. Since this situation obtains in ordinary breeds of fowl, it has been natural to look for an endocrine causation of hen-feathering in those strains where the male has a plumage similar to that of the female. The prompt assumption of male plumage by the castrated Sebright,[3] which is normally hen-feathered, seemed at first to supply definite evidence of an endocrine causation for hen-feathering in the male. But cross-transplantation of testes in Sebright and Leghorn fowls[4] showed that the testes of hen-feathered Sebrights produce no special hormone different from that of cock-feathered Leghorns; and the experiments to be reported at this time indicate that follicles from a hen-feathered strain and those from a cock-feathered strain may produce their respective types of feathers when grown side by side and subjected to the same endocrine environment.

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[1] (a) By gonadectomy experiments, H. D. Goodale, *Carnegie Inst. of Wash. Publ.* 243, 1916; L. V. Domm, *J. Exp. Z.*, 1927, xlviii, 31. (b) By thyroid feeding. H. B. Torrey and B. Horning, *Anat. Rec.*, 1923, xxiv, 395. (c) By gonadal transplantation, Pézard, Sand et Caridroit, *Compt. rend. Soc. Biol.*, 1925, xcii, 427. (d) By skin transplantation, Danforth and Foster, PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 75.

[2] Lillie, F. R., *J. Exp. Zool.*, 1927, xlviii, 175.

[3] Morgan, T. H., PROC. SOC. EXP. BIOL. AND MED., 1915, xiii, 31; *Carnegie Inst. of Wash. Publ. No.* 285, 118 pp., 1919.

[4] Roxas, H. A., *J. Exp. Zool.*, 1926, xlvi, 63.

The experiments consisted in transferring pieces of skin of the saddle region (where plumage dimorphism, when present, is most marked) from one newly hatched chick to another and later comparing the feathers produced by the donor, the host and the transplant. The following cases may be cited. They should be viewed in the light of the finding that where only ordinary breeds are involved in such transplants the grafts invariably produce feathers corresponding to the sex of the host, irrespective of the sex of the donor.

1. A white Leghorn cock, with good male plumage at maturity, supported a Campine graft which produced numerous feathers of the female type and a few with a narrow marginal fringe such as occasionally appears in normal hen-feathered Campines. The feathers on the graft were comparable to those of control Campine males of the same age. Grafts from Rhode Island Reds and Minorcas on similar Leghorn males have regularly produced a full complement of male feathers.

2. A bantam male with a moderate grade of cock-feathering was grafted with skin from a hen-feathered bantam female. All the feathers produced by this graft were of the female type, with a sharp line of demarcation between them and those of the host.

3 and 4. Exchange grafts were made between 2 bantam males, one of which developed a full male plumage, while the other developed a plumage of a somewhat restricted, intermediate type. In both cases the feathers produced by the graft corresponded very closely to those of the *donor*.

It is evident that in these cases the differential factor was inherent in the skin—presumably in the feather follicles themselves—rather than in circulating fluids of the host, which were distributed alike to his own and to the grafted skin. This points to the conclusion that the difference between the hen-feathered and the cock-feathered male fowl is primarily a difference in reactivity of the skin, the "thresholds" being different in the 2 types. This pecularity of the skin is obviously hereditary. It is possible that there are also hereditary endocrine differences which influence the type of plumage in the fowl, but they have not been apparent in these experiments.

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Cl. Botulinum Type D Sp. N.

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From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.

During the past year the laboratory has received several specimens of botulinus-like organisms through the courtesy of Drs. P. J. du Toit and E. M. Robinson, Onderste-Poort, Pretoria, South Africa. Although these cultures differ in many ways from those isolated in this country, one strain secured from lamziekte carrion deserves special consideration.

The fluid portion of the original culture was toxic for mice. The antitoxic sera Type A, B and C failed to protect the animals against this poison irrespective of the fact that the symptoms and lesions were in every respect typical for botulism. A highly potent toxin was obtained in beef heart-peptic digest mash (1 cc. = 20,000 guinea pig M.L.D.) and Hibler's brain medium. Protection experiments with the known type antitoxins and a serum supplied by Dr. E. M. Robinson known as *"Cl. parabotulinum bovis antiserum"* were made on guinea pigs.

The results clearly indicate that the toxin of one of the South African strains of *Cl. botulinum* is different from either *Cl. botulinum* Type B and C or *Cl. parabotulinum* Type A and B.

In pure culture the organism resembles the anaerobe described by Theiler and Robinson.[1] It is a long, slender, Gram-positive, unusually pleomorphic rod which is actively motile under strictly anaerobic conditions. In egg-medium (Rettger) numerous subterminal closteridium-like spores are formed, while in beef heart and other media spores are rarely observed. Rhizoid, non-hemolytic surface colonies appear on properly prepared glucose blood plates. Growth is good and a small amount of gas is formed in beef heart, egg and milk media provided a vaseline seal is used. The proteolytic properties are low (Sörensen's figures 1st day 0.5, 14th day 4.0), about the same as the non-ovolytic Type B and slightly higher than the Type C strains.

The cultures have a strong odor of butyric acid. Indol is not produced. Glucose, levulose, sucrose, lactose, maltose, glycerine, inosite and galactose are fermented when the test medium is composed of 0.3% Liebig's extract, 0.5% Difco peptone and 1% carbohydrate. No gas is liberated, but the change in pH is marked. In many of the sugars a final pH after 4 days incubation is 4.6 to 5.0. *Cl. botulinum* Type D is the only strain thus far encountered which fails to produce gas from carbohydrate. It also differs from other strains in its action on sucrose and lactose. Toxic and non-toxic variants may be isolated from fluid cultures.

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[1] Theiler and Robinson, *Z. f. Infektionskr. d. Haustiere*, 1927, xxxi, 165; 11th and 12th Reports of Director of Veterinary Education and Research, Dept. Agri., Union of S. Africa, 1927, II, 1099.

The susceptibility of various animals for the toxin is briefly as follows: Chickens and dogs are non-susceptible to subcutaneous injection of 100,000 guinea pig M.L.D.'s; 5 guinea pig M.L.D. subcutaneously are fatal to a rabbit in 72 hours; 1,000 M.L.D. for a rat in 7 days; 100,000 M.L.D. for a pigeon in 24 hours. Contrary to the observation of Theiler, mice are susceptible; a toxin which was fatal for guinea pigs in a dilution of 1:20,000 fatally poisoned mice in a dilution of 1:50,000. The ratio of the M.L.D. by subcutaneous injection to the M.L.D. *per os* is 1:1,000 for guinea pigs, 1:200 for mice and >1:3,000 for rabbits. Suitable suspensions of the *Cl. botulinum* Type D are not agglutinated by any of the type sera available in this laboratory but are readily clumped by a homologous antiserum.

The anaerobe isolated from lamziekte carrion is a non-ovolytic and non-sarcolytic organism which produces a highly potent neurotoxin. Pharmacologically the poison acts on small laboratory animals like the botulinum toxin. It is not neutralized by any of the known Type A, B and C antitoxins. The bacterium should therefore be designated *Cl. botulinum* Type D (Theiler and Robinson[1]) and should be definitely separated from the ovolytic *Cl. parabotulinum* Type A and B.

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Susceptibility of Macacus Rhesus Monkeys to Botulinum Toxin Type B, C and D.

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Thus far no cases of botulism due to *Cl. botulinum* Type C have been reported and nothing definite concerning the susceptibility of man to this toxin is known. On the other hand since the great susceptibility of monkeys to *Cl. parabotulinum* Type A toxin is now established through the studies of Dack and Wood[1] it appeared not unlikely that comparative tests on these animals with non-ovolytic and non-sarcolytic strains might permit of certain deductions concerning the liability of man to botulism poisoning by the ingestion of Type C and D toxins.

Such tests became imperative since Bengtson[2] considers the monkey very susceptible to the toxin of *Cl. botulinum* Type C and the reports of Theiler and Robinson[3] contain no information concerning the effect of Type D toxin on apes. A series of monkeys was therefore injected or fed by stomach tube with varying doses of different toxins.

The tests confirm the early observations of van Ermengem[4] and Römer and Stein[5] that monkeys are very susceptible to *Cl. botulinum* Type B toxin (produced by a strain recently isolated from a ham in Germany) by feeding. In the animal poisoned by this type, toxin was demonstrated in the blood and throughout the entire intestinal tract but not in the brain. Although readily intoxicated by subcutaneous injections with Type C and D toxin the *Macacus rhesus* resist very large doses of the same poison *per os*. The first visible symptoms in the animals injected subcutaneously appeared only 60 to 90 minutes before death. Since the number of the experiments here presented is small, one may merely conclude that the susceptibility of man to Type D and in all probability to Type C toxins is low.

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[1] Dack and Wood, *J. Infect. Dis.*, 1928, xlii, 209.

[2] Bengtson, *Pub. Health Rep.*, 1922, xxxvii, 164.

[3] Theiler and Robinson, 11th and 12th Reports, Director of Vet. Educ. and Res., Union of S. Africa, Dept. of Agri., 1927, II, 1099; *S. African J. Science*, 1925, xxii, 141; *Z. f. Infektionskr. d. Haustiere*, 1927, xxxi, 190.

[4] Van Ermengem, *Z. f. Hyg. u. Infectionskrankh.*, 1897, xxvi, 1.

[5] Römer and Stein, *Arch. f. Ophth.*, 1904, lviii, 291.

4154

Experimental Food Poisoning in White Mice with Heat Stabile Paratyphoid Poisons.

J. C. GEIGER AND K. F. MEYER.

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.

A study of human outbreaks of food poisoning indicates that a severely irritant substance, probably produced by representatives of the paratyphoid-enteritidis group and other bacteria when growing on foods rich in protein, exerts its action on the alimentary tract. A number of workers (Savage and White,[1] Branham, Robey and Day[2] and others) have sought for experimental evidence of such an irritant. Most of the experiments were inconclusive or merely suggestive on account of the apparent insensitiveness of the animal in contrast to the human intestinal tube. Following the oral administration of living or dead paratyphoid bacilli a definite pathological reaction was either absent or unobtrusive; and delayed (5 to 10-14 days, Branham and Day) symptoms were produced unless a general infection was eventually set up. However, in this connection it must be recalled that Gärtner[3] as early as 1888 observed significant clinical and pathological manifestations in mice fed with meat previously contaminated with *B. enteritidis* and heated for 1 hour at 100°, A casual perusal of the literature indicates that the experiments of Gärtner have never been repeated on a large scale or with strains which have been recently isolated from outbreaks of food poisoning. The toxicity of these organisms is usually tested by parenteral injections of filtrates or boiled cultures and not by systematic feeding experiments (Bahr and Dyssegaard[4]). These considerations suggested a renewed investigation. Although the studies are still in progress a number of interesting observations deserve wider recognition.

Under suitable experimental conditions which will be detailed elsewhere certain strains of *B. aertrycke* produce after an incubation time of 3 to 4 days at 37° C. a highly potent enterotropic poison. The active principle is present in filtrates in very potent concentrations, in heated whole fluid cultures. It acts both by intraperitoneal injection and by feeding with the aid of a catheter, or when mixed with bread. The production of symptoms and characteristic anatomical changes was practically universal when a particular strain in a special medium was heated for 10 to 60 minutes at 100° C. or subjected to a temperature of 240° for 10 minutes in the autoclave. Administered by catheter the sterile, heated poison was fatal to mice weighing approximately 20 gm. in some instances in 0.05 cc. and usually in 0.1, 0.2 and 0.5 cc. amounts. The heated media treated in an identical manner as the cultures are not poisonous.

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[1] Savage and White, Med. Res. Council, Special Rep., Series 91, 1925, 111.

[2] Branham, Robey and Day, *Arch. Path.*, 1928, v, 742; *J. Bacteriol.*, 1928, xv, 36.

[3] Gärtner, *Correspondenz-Blatter d. allg. arztl. Vereins v. Thüringen*, 1888, xvii, 590.

[4] Bahr and Dyssegaard, *Centralbl. f. Bakteriol.*, 1927, cii, 268.

TABLE I.

Fed 0.5 cc. of fluid culture of *B. aertrycke* grown 4 days—then sterilized by	No. of mice fed	No. of deaths	Elapse of time from feeding to death
1. 10 minutes at 100°	5	5	10-13 hours.
2. 60 minutes at 100°	5	3	3-13 hours.
3. Autoclaved 10 min. at 240°	5	5	1 in 7 and 4 in less than 24 hours.

Approximately 30 minutes after oral administration, the mice exhibit a quiet attitude; in from 60 to 180 minutes they may show uneasiness, excitement and spasms, roughened coat, labored and accelerated respiration Later, the eyes are closed by a crusty exudate. Shortly before death, which occurs in from 6 to 12 to 24 hours, general weakness is not uncommon and the motionless animals are flattened out on the bottom of the cage. In approximately 50% of the poisoned animals the anus is soiled with fluid intestinal content or a plug of glassy mucous. On autopsy the distended abdomen is soft, and, on opening reveals a markedly distended duodenal loop and deeply injected moist but empty coils of the small intestines. The stomach, filled with a small amount of food, is frequently distended by the same bile tinged or some blood streaked mucous which is present in the ballooned duodenal loop. The mucosa is always injected and sometimes covered with petechia and small erosions. Soft or fluid chyme is found in the large intestines; the jejunum and ileum contain as a rule flakes of clear yellowish mucous. The spleen may be slightly enlarged and the liver is hyperaemic. In some instances both pleural cavities contain a blood tinged or straw colored fluid (0.2-0.5 cc.); the lungs are bright red and collapsed. Cultures from the heart blood, spleen and liver remain sterile, while those from the intestinal content fail to reveal representatives of the paratyphoid group. The distended duodenum, the hyperaemic jejunum and ileum and the pleural effusion are pathognomonic for experimental food poisoning in white mice. In the experience of the writers deaths occurring later than 48 hours after feeding are probably due to other causes than the paratyphoid poison which acts primarily on the duodenum and stomach but may extend its reaction down the intestines beyond the duodenum. Rabbits, guinea pigs and cats fed double lethal mouse doses by weight of the poison may present transitory and indefinite symptoms from which they rapidly recover. Their blood collected 2 weeks after the feeding of the poison usually agglutinates the paratyphoid bacilli in low dilutions.

Symptoms of food poisoning have been produced in a macacus monkey by feeding 10 cc. of a potent poison and its blood had an agglutination titre of 1/640 2 weeks later.

4155

Electrolytes of Saliva Under Normal and Pathological Conditions.

H. BECKS. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco.

The growing perception of the close relations of saliva to the teeth and the surrounding tissues led to systematic analyses of the mixed saliva in order to obtain normal or standard values for the electrolytes and thus a foundation for the study of this secretion under pathological conditions. The examinations of the physiological resting saliva were made on persons with absolutely intact teeth and normal gum tissues, and, who were known not to have been sick within the last 3 or 4 years. In all cases the saliva has been analyzed as to its content of cations and anions, namely Cations: K, Ca, Mg, Na; Anions: Cl, P, (*i. e.*, PO_4), CNS, as well as the H-ion concentration (pH). The CO_2 capacity and viscosity.

1. It was found that under physiological conditions the excretion of cations and anions diminishes, with the exception of potassium (between 20 and 40 years) but noticeably increases with an advance in age.

2. By an arithmetical average for the different saliva components the following normal standard values have been determined:

HCO_3=93.16 mg.% (Determination according to van Slyke).
pH= 7.15 (Determination with the Quinhydronelectrode).
K=55.89 mg.% (Determination according to Kramer Tisdall).
Ca=12.13 mg.% (Determination according to Kramer Tisdall).
Mg= 1.29 mg.% (Determination according to Kramer Tisdall and Tschopp).
Cl=74.68 mg.% (Determination according to Tschopp or Bang).
PO_4=13.16 mg.% (Determination according to Tschopp or Bell and Doisy).
CNS=30.9 mg.% (Colorimetric method).

3. Electrolyte composition of the saliva during one day:

a. Potassium and Sulfocyanide show the lowest values in the morning before breakfast, and then increase slowly, having the highest percentage in the afternoon; overnight the values diminish until the lowest point is again reached.

b. Calcium and Chloride contents vary in opposite directions to the potassium and sulfocyanide, being highest in the morning and overnight. During the day they remain nearly constant.

c. In comparison to the other cations the amount of magnesium is very small, showing the lowest values before breakfast in the morning. About noon it reaches the highest point and then gradually decreases. It seems that the magnesium is found inversely proportional to sodium; about its properties and functions in the body however little is known.

d. Phosphorus (PO_4), HCO_3 and pH are least constant; no constant values could be found as for the electrolytes mentioned under a-c.

4. The H-Ion concentration of the saliva collected from the parotid differs fundamentally from the mixed saliva of the same individual. In fact, the parotid saliva is primarily acid, while the secretion of the sublingual and submaxillary glands falls into the alkaline range.

5. A Calcium compound which is negatively charged and readily absorbed by positively charged absorbents is present in the saliva. The compound is probably the main factor in the formation of tartar.

6. Through a special mode of experimentation it is now proven that the saliva is not a saturated solution of $CaCO_3$ and $Ca_3(PO_4)_2$.

7. In the majority of the tooth pastes now available on the market readily soluble Ca salts have been demonstrated. These salts increase temporarily at least the Ca content of the saliva and lead to retention and depositions in the interdental spaces. Other substances of the pastes exert an absorbing action on the negatively

charged Ca compound of the saliva and accelerate the precipitation.

8. Examinations of the mixed saliva collected from patients with pyorrhea have shown that in the majority of instances the composition reveals a marked increase of the cations over the values established for the individuals with mouths free from pyorrhea. In particular the amounts of K and Na are higher while correspondingly the other values are decreased. The Ca and PO_4 percentages are reduced approximately 25% and the amount of Mg even 40%.

9. Examination of the saliva during pregnancy has shown that the Mg is increased (in contrast to pyorrhea) and the Ca content reduced to approximately 50% of the normal value.

4156

Studies on the Heat Resistance of Spores of B. Subtilis.

O. B. WILLIAMS. (Introduced by Karl F. Meyer.)

From the Division of Bacteriology, University of California Medical School, San Francisco.

This report presents in summary form the results of a study of the survival time of *B. subtilis* spores produced under a wide variety of nutritive and environmental conditions and exposed to high temperatures. The basic resistance has been defined as the resistance of a suspension of a definite density, produced and tested under certain standard conditions. The basic resistance of the strain employed was 6 minutes at 105° C., 14 minutes at 100° C., and 35 minutes at 95° C. Most of the determinations were made at 100° and 95°.

Pronounced deviations from the basic resistance have been produced by changing the nutritive substratum. The kind of peptone used has been found to be significant, but the resistance appears to have been independent of the concentration of the peptone.

Spores of diminished resistance were formed in all digest media tested with the exception of casein digest, which yielded spores of an enhanced resistance. The explanation of this probably rests on the difference in phosphate content of these media. Very resistant spores were formed in isoelectric gelatin. Evidence which has been acquired indicates that this was probably due to the low salt content of this medium. Infusions of vegetable matter were found to be excellent media. Spores of high resistance were formed in these media in all cases. Slightly more resistant spores were formed in liquid media than in solid media. Cultivation in the presence of a suitable concentration of either phosphate or magnesium resulted in the formation of spores considerably more resistant than the normal spores. Spores slightly more resistant than normal were formed in the presence of calcium or iron, and also by cultivation in physiologically balanced solutions. The increase in these cases, however, was not pronounced. A number of inorganic salts were found to be without influence on the resistance of spores produced in media to which they had been added. Media enriched by the addition of certain organic substances were found to yield spores greater in resistance than the normal. Of especial significance in this connection may be mentioned available carbohydrates and certain organic and amino acid radicles.

No correlation was apparent between the extent of growth or sporulation and the resistance of the spores produced. The initial reaction of the medium in which the spores were formed was found to be without influence. The temperature of cultivation, however, was found to be of considerable significance, the higher temperatures resulting in spores of an enhanced resistance. Cultivation in a medium with the surface tension reduced by the addition of bile resulted in spores of normal resistance. It was found that the time necessary to sterilize a suspension at a given temperature increased, as the concentration increased. With uniform concentrations the time necessary to sterilize decreased as the temperature increased.

No effect was apparent as a result of heating in a solution of increased density, or in tubes of different lengths. Exposure of the cultures to X-rays before incubation had no effect on the resistance of the spores produced. Evidence has been acquired which emphasizes the necessity of using strained suspensions in heat resistance tests. Irregular and erroneous results will be obtained unless this precaution is observed. Confirmation of the results of previous workers on the disinfecting action of the hydrogen and hydroxyl ions has been obtained.

Desiccated spores exhibited a resistance in excess of that exhibited by young, moist spores. Spores stored for an equal length of time in a moist condition were not, however, appreciably changed in resistance. The course of mortality, as determined by quantitative plating at various time intervals, was not of such a nature that it could be described by the equation for a first order chemical reaction. Dormancy, either of heated or unheated spores, was not exhibited by the culture used in this study.

The resistance of the spores was increased by a process of selection of resistant cells. Efforts to increase the resistance by cultivation in media which regularly yielded spores of an enhanced resistance were not successful. Attempts to produce spores in any appreciable quantity in synthetic media prepared from highly purified chemicals met with failure.

Evidence gleaned from the literature and accumulated during the progress of this work supports the idea that the cause of death in cells exposed to a high temperature is the coagulation of bacterial protein. Conditions which render protein more difficult to coagulate consequently result in an increased resistance to heat. The water and the ash content of the cell appear to be especially important in this connection. However, cultivation under certain nutritive conditions which do not appear to be intimately related to either the water or ash of the cell has invariably resulted in the production of resistant spores.

4157

An Endeavor to Adapt a Trypsin Susceptible Bacteriophage to the Action of Trypsin.

E. W. SCHULTZ and E. A. GREEN.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

It is well known to students of the d'Herelle phenomenon that bacteriophages, with rare exceptions, resist the action of trypsin for indefinite periods of time. Recently, one of us[1] described the trypsin susceptibility of 2 races of staphylococcus bacteriophages; one a monovalent, the other a polyvalent race. These 2 races were found to be highly susceptible to the action of trypsin, complete inactivation following an exposure to this enzyme within 48 hours at incubator temperature. In view of the difference of opinion entertained by various investigators on the question of whether bacteriophages actually possess powers of adaptation, a property indicative of life, it occurred to us that this susceptibility to tryptic activity might possibly serve as a basis for determining further the capacity of bacteriophages to adapt themselves to deleterious or inhibiting agents.

Two methods of arriving at an answer were employed. In one series of experiments, the bacteriophage was exposed to the action of trypsin in the presence of lysable staphylococci. That is, the customary procedure for producing bacteriophage suspensions was employed, with the exception that the Martin's broth contained active trypsin.* After complete lysis had been attained the limpid liquid was filtered through a Chamberland candle (L 3). A few drops of the filtrate were then added to a fresh suspension of staphylococci in trypsinized broth and so on in series. Any adaptation that might be effected could be due either to an adjustment on the part of the bacteriophage, or, should the bacteriophage in reality represent a product of the bacteria themselves, to an adjustment on the part of the staphylococci producing it.

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[1] Schultz, E. W., Proc. Soc. Exp. Biol. and Med., 1928, xxv, 280.

In the other series, the bacteriophage was exposed to the action of trypsin immediately following filtration of the lysed culture through a Chamberland filter.* The exposure to trypsin therefore occurred in the absence of lysable staphylococci. After a variable period of exposure to the action of the trypsin, generally just before complete inactivation of the bacteriophage suspension, a few drops of the suspension were transferred to non-trypsinized Martin's broth containing an appropriate number of staphylococci for the regeneration of the lytic principle. The filtrates of these lysed cultures were again trypsinized to the point of almost complete inactivation; regenerated by passage on staphylococci in ordinary Martin's broth and so on in series. Any adaptation that might be realized would be more directly attributable to a shift in the properties of the bacteriophage itself. In both series suitable controls were always set up. Both series were repeated several times and were altogether carried on over a period of nearly a year.

The bacteriophages presumably undergoing an adaptation were tested at intervals for evidences of increased resistance to the action of trypsin. These measurements were perforce made in terms of the normal, "unadapted," bacteriophage. The criterion of adaptation consisted in noting any difference in the length of time that the bacteriophage, supposed to be undergoing adaptation, remained active after the normal bacteriophage, in the same set-up, had become definitely inactive. In carrying out these comparison tests every effort was made to secure reasonably comparable conditions for both the normal and "adapted" bacteriophages. The activity of these suspensions was determined at short intervals up to the time of complete inactivation. Suspensions were not considered completely inactive unless 5 or 6 serial passages on susceptible staphylococci failed to elicit any evidence of an active principle.†

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* In preparing the trypsinized broth for the first series of experiments, also in trypsinizing the filtered bacteriophage suspensions in the second series, increasing quantities (1-4%) of powdered trypsin were added to the broth, or bacteriophage suspension, and after allowing the mixture to stand for about a half hour, was filtered through paper and finally through an L 3 Chamberland filter. The experiments were all carried out at incubator temperature and the activity of the trypsin was checked in each experiment by means of a capillary tube of heated egg-white immersed in the liquid. Several brands of trypsin were employed during the course of the work. No natural bacteriophages active for staphylococcus could be isolated from the trypsin preparations employed in the studies.

The results obtained indicate that some increase in resistance to trypsin is acquired by the bacteriophage in the course of a number of serial exposures to suitable concentrations of trypsin. As nearly as could be determined, this increase in resistance was approximately the same for bacteriophages exposed to the action of trypsin in the absence of lysable staphylococci, as for bacteriophages regenerated at the expense of staphylococci, multiplying in the presence of an active trypsin. In neither of the series was the increase in resistance marked. Whereas the normal bacteriophages generally become definitely inactive by the end of 48 hours, the bacteriophages previously exposed to the action of trypsin remained definitely active at the end of this time and frequently continued to remain so for some days longer, provided retrypsinization was not resorted to in the meantime. Retrypsinization at the end of the 48 hour period always resulted in an inactivation of these more resistant suspensions within the next 24 hours. This may be explained on the ground that although some tryptic activity still manifests itself at the end of 48 hours, as judged by the continued digestion of egg-white in the capillary tube immersed in the bacteriophage suspension, the activity of the trypsin undoubtedly rapidly dwindles after this period. For this reason one cannot count bacteriophagic activity much in excess of 48 hours as evidence of further increase in resistance, unless retrypsinization of the suspension has been resorted to. Consequently, some increase in resistance could be elicited, *i. e.,* if we may make this deduction from a careful comparison with corresponding normal bacteriophages. Though we endeavored by continuing the process to render the bacteriophages completely resistant to the action of trypsin we failed to get it beyond the point where merely a well defined difference could be recognized. We are not at all sure that the shift in property may in reality be regarded a true adaptation of the bacteriophage. Two such suspensions when tested some months later no longer exhibited the relative increase in resistance to the trypsin.

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† In this connection we invite attention again to the delayed lysis, previously reported by one of us,[1] in which clarification of the bacterial suspensions may not become obvious until some time after the period of logarithmic bacterial growth has ceased. We have been unable to elicit any evidence that the staphylococci undergoes spontaneous lysis, even when grown in trypsinized broth. The phenomenon is undoubtedly determined by a very low initial concentration of the bacteriophage. Such tubes when observed at room temperature first clear at the top and the clarification then gradually extends downward to the bottom of the tube.

4158

Inactivation of Staphyloccus Bacteriophage by Methylene Blue.

E. W. SCHULTZ AND A. P. KRUEGER.
From the Department of Bacteriology and Experimental Pathology, Stanford University.

Recently, one of us[1] described the trypsin susceptibility of 2 races of staphylococcus bacteriophages, a property which stands in striking contrast to the marked resistance offered to this enzyme by bacteriophages generally. It occurred to us to compare these 2 races of bacteriophage with a number of other races in our possession, as to their susceptibility to other chemical agents, particularly dye stuffs. Various dyes, including Carmine, Congo Red, Methyl Red, Neutral Red, Methyl Green, Brilliant Green, Brilliant Cresyl Blue, Trypan Blue, Basic Fuchsin, Crystal Violet, Gentian Violet, Aniline Violet, Orange G, Eosin B, Bismark Brown, and Methylene Blue,* were added in relatively high concentrations to bacteriophage filtrates, which were then kept at incubator temperature, in part at room temperature, for 24 hours or longer and thereafter tested for lytic activity. No appreciable influence on any of the bacteriophages was noted, with the single exception of the effect produced on the 2 staphylococcus bacteriophages by methylene blue. These particular bacteriophages were completely inactivated within 6 to 12 hours by concentrations of the dye as low as 0.002%. Five serial passages on susceptible staphylococci failed to elicit any evidence of residual active principle. Believing that the action might be due to some impurities in the methylene blue, we then employed the dye after careful recrystallization. The same results were realized. Eight other bacteriophages, including races of anticoli, antidysentery, antityphoid, and antiproteus bacteriophages did not appear to be influenced in the least by the dye. The susceptibility to methylene blue appears therefore to be peculiar to the 2 races of staphylococcus bacteriophages examined for this property. This susceptibility may possibly extend itself to bacteriophages active for other non-intestinal bacteria as well as to other antistaphylococcus bacteriophages. The mechanism of the inactivation is not exactly clear, but will be studied further. We have been able to satisfy ourselves that it is not in any way related to pH changes induced by the dye in the medium.

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[1] Schultz, E. W., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 280.

* Mercks Medicinal Methylene Blue.

4159

Spontaneous and Forced Dissociation of Brucella Abortus (Bang).

B. S. HENRY. (Introduced by T. D. Beckwith.)

From the Division of Veterinary Science, University of California.

In making a study, with Dr. J. Traum, on the causes of discrepancies occasionally encountered in testing cattle sera for *Br. abortus* agglutinins, it was noticed that different lots of antigens from certain strains gave varying reactions with the same sera. It was necessary to conclude that the antigen varied because all negative sera gave similar reactions on such lots of antigen and these same sera gave only definite negative results with antigens from many other strains of *Br. abortus*.

To determine the character of the variation mentioned above, glucose-glycerine agar plates were dallied with suspensions of the growth to be used for antigens. At the end of a 4-day incubation period, colonies of 2 distinct and constant types were present. One is the usual *Br. abortus* colony, moist, clear and only slightly granular. By transmitted light, these colonies show a blue-green florescence. The second type of colony is opaque, very definitely granular, and grows more rapidly than does the clear type. Subcultures from the clear type are readily suspended in salt solution and remain suspended for several days. The opaque type, on the other hand, is suspended with difficulty and spontaneously agglutinates within 24 to 48 hours. The subcultures of the opaque type have remained free of clear type colonies through several transplant generations. The clear cultures, however, continue to produce an occasional opaque colony even after several platings.

Plates made from 23 stock strains of *Br. abortus* showed none of these strains to be entirely free of both types, although the ratio of opaque to clear colonies varied over a wide range.

Before the above-mentioned spontaneous changes were noted, the author had been able to produce opaque colonies from typical *Br. abortus* cultures by growing the organisms in glucose-glycerine broth to which had been added 10% of serum from rabbits immunized against *Br. abortus*. The changes which occurred in these cultures were similar to those occurring spontaneously, but were more complete. All colonies appearing on plates made from 2 week old immune broth cultures were of the opaque type in the 6 strains used.

As the 2 types of colonies occurring spontaneously, as well as those obtained by the use of homologous immune serum, agree with *Br. abortus* as to morphology, staining reaction and carbohydrate fermentations, and as, in at least one strain, they both produce *Br. abortus* agglutinins when inoculated into rabbits and guinea pigs, it seems certain that dissociation is responsible for the 2 types. The terms "R" and "S" have been intentionally omitted until the results of virulence tests now in progress have been determined.

4160

Mechanism of Actions and Toxicity of Nitroprusside.

CHAS. C. JOHNSON. (Introduced by P. J. Hanzlik.)

From the Department of Pharmacology, Stanford University School of Medicine.

The actions and toxicity of sodium nitroprusside ($Na_2Fe(CN)_5 NO2H_20$) have been investigated periodically during the past 42 years without, however, definite establishment of the mechanisms on which the actions depend, or indications of its possible usefulness. The original claims of L. Herrmann[1], of his pupil Davidsohn[2], and of Arntz[3] and Cromme[4], that the actions of nitroprusside depended upon liberated hydrocyanic acid suggested the possible use of the compound as a respiratory stimulant. The actions of the compound, therefore, have been re-investigated along different lines with the following results.

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[1] Herrmann, *Arch. für Physiol.*, 1886, xxxix, 419.

[2] Davidsohn, *Diss.*, Königsberg, 1888, 34.

[3] Arntz, *Diss.*, Kiel, 1897, 24.

[4] Cromme, *Diss.*, Kiel, 1891, 16.

The marked symptoms claimed by previous investigators, namely sustained, intense respiratory stimulation, followed sometimes by tremors and convulsions in all species, and accompanied by nausea and emesis in mammals and birds, have been confirmed. The odor of cyanogen *post mortem,* following large fatal doses only, has been confirmed, but chemical tests for cyanogen in the blood and tissues have proved inconclusive. Other tests were applied because small and non-fatal doses (2 mgm. per kilo or less hypodermically and about 0.1 to 0.2 mgm. per kilo intravenously), still gave profound effects without demonstrable cyanogen. These effects in narcotized and operated animals consisted of marked respiratory stimulation and a marked and sustained fall of blood pressure accompanied by increases in volume of peripheral organs and diminution in cardiac volume and contractions with increase or without change in rate. The circulatory actions were practically identical with those of sodium nitrite, but opposite to those of cyanide, in the same animals. A nitrite-type of action was further indicated from the facts that the vasomotor center still responded to reflex and asphyxial stimulations, the smooth muscle and sympathetic endings of the blood vessels were responsive in the usual way to epinephrine and the smooth muscle of excised organs was typically depressed. These actions might be expected from the nitroso (NO) group in the nitroprusside, and the respiratory stimulation would result from the depressor action, rather than from the alleged liberation of cyanogen.

Further evidence of a negative character bearing on the cyanogen mechanism was obtained with incubated pigeon corpuscles. The respiration of these cells was promptly inhibited by sodium cyanide (equal to 1:600,000 of (CN), but not by sodium nitroprusside in concentration theoretically capable of liberating 10 times this concentration of cyanogen, or about 16 times the concentration of nitroprusside assumed to be present in blood after definitely effective doses (0.1 mgm. per kilo intravenously). One hundred times the concentration of nitroprusside, however, did cause some depression of respiration in the corpuscles. The fermentation activity of yeast was unaffected by nitroprusside.

Provisionally, it appears that the actions of nitroprusside, in definitely effective but not fatal doses, do not depend upon the liberation of the cyanogen group. Since the circulatory and smooth muscle actions resemble those of the nitrite group, nitroprusside may have some therapeutic usefulness.

4161

Calcium and Phosphorus Balances in Rats During Period of Pregnancy and Lactation.

HAROLD GOSS AND CARL L. A. SCHMIDT.

From the Division of Biochemistry, University of California Medical School, Berkeley.

Rats were placed on a constant diet and periodic collections of excreta were analyzed in order to follow the calcium and phosphorus balances during the period of pregnancy and lactation. The diet was made alkaline by the addition of calcium carbonate. All of the necessary factors were included in the diet.

After the onset of pregnancy and prior to parturition, the animals stored calcium and phosphorus in excess over that estimated to be contained in the litter at birth. During the period of lactation, the input of both calcium and phosphorus increased markedly, but this increase was not sufficient in most cases to account for the storage of these elements by the young. The animals were consequently in negative balance. Balances were carried out during 2 successive periods of pregnancy and lactation.

Other experiments to determine the effects of variations in the reaction of the diet are in progress.

4162

Body Temperature Regulation. Effects of Labyrinth Destruction on Tonus and Body Temperature in Rabbit.

LILLIAN M. MOORE.

From the Department of Physiology, University of California, Berkeley.

Labyrinth destruction in the rabbit as well as in other animals produces a marked temporary and a less marked permanent decrease in muscular tonus. If body temperature is in any way dependent upon the heat production of tonic contractions these characteristic tonus changes should be accompanied by equal and parallel changes in the body temperature.[1]

Four or 5 entirely successful operations have been performed with identical post-operative symptoms. The tonus modifications are immediately evident, but the development of the hypotonia continues for about 2 hours, during which time the animal lies on its ventral side (an unusual position for a rabbit) in a completely relaxed condition, unable to maintain any antigravity posture, but permitting the elicitation of reflexes. This condition, however, is transient and within 2 to 3 hours the tonus has increased until a sitting and finally a standing position is regained. The animal then makes occasional progressive movements, but with evidence of great muscular weakness.

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[1] Moore, L. M., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 48.

The temperature changes follow the same course. There is an immediate fall, in the example given, from a normal of 39.5° C. to 35.4° C. followed by a further gradual decrease to 34.6° at the time of greatest tonus loss. As the tonus again increases, the temperature rises until it reaches 38.3° C. about 10 hours after the operation. It later drops one degree and remains near 37.5° C. until the rabbit is killed.

One example, with several readings omitted for brevity, is given.

Time	Temp. of rabbit in ° C.	Tonus symptoms
7/19/28— 9:30 a. m.	39.5	
10:00 a. m.	destruction of right labyrinth	
10:30 a. m.	destruction of left labyrinth	
11:00 a. m.	35.4	Very relaxed
11:15 a. m.	34.9	Lying on ventral side.
11:45 a. m.	34.6	Marked hypotonia.
12:00 m.	34.8	
1:00 p. m.	36.3	Sitting erect.
4:20 p. m.	37.8	Occasional walking.
7:45 p. m.	38.3	
7/20/28— 8:00 a. m.	37.4	Muscular weakness.

The hypothermia in these cases cannot be due to interference with any temperature regulating mechanism in the brain, but is entirely an indirect result of the lowered tonus characteristic of labyrinth destruction. The relation which seems to exist between muscular tonus and body temperature would appear to be very significant, and may be found to account for the constant and relatively high temperature of birds and mammals.

4163

Body Temperature Regulation, Hypothermia Due to Lesions Involving Vestibular Nuclei of Rabbit.

LILLIAN M. MOORE.

From the Department of Physiology, University of California, Berkeley.

In a previous communication it was shown that localized lesions in the midbrain produce a fall of body temperature from a normal of 39-40°C. to 28-26° C. within about 5 hours.[1] Accompanying the hypothermia is a gradual decrease in muscular tonus and at 26° C. almost complete atonia. The following example will illustrate the rate of change in both:

Time	Temp. of rabbit in ° C.	Tonus symptoms
9:30 a. m.	40.0	
10:45 a. m.	Injection of chromic acid	
11:30 a. m.	35.5	Relaxed
11:50 a. m.	34.0	Relaxed
12:00 m.	32.5	.Marked hypotonia, no reflexes.
12:20 p. m.	32.0	
1:30 p. m.	30.0	
3:00 p. m.	29.0	
3:30 p. m.	28.0	
6:00 p. m.	.28.8	Complete atonia.
9:00 p. m.	26.0	Complete atonia.

The similarity between the temperature and the tonus changes suggests the dependence of the former upon the latter. This suggestion is strengthened by the fact that the effective lesion is very limited in extent and exact in location and always involves the posterior longitudinal bundle with its fibers from the vestibular nuclei. This tract is probably partially responsible for normal tonus, because of the relationship of most of its fibers to the vestibular nuclei which are necessary for normal tonus. These nuclei seem to maintain an inhibition to the normal myotatic inhibition within the muscle itself, and their dysfunction or perhaps a break at any point in the reflex arc, as for example in the posterior longitudinal bundle, may release the tonic inhibition in the muscle with subsequent atonia. The atonia causes a decrease in heat production probably .of sufficient magnitude to bring the body temperature down to the abnormally low level attained in these experiments.

An attempt was made to destroy the vestibular nuclei by chromic acid injections similar to those made in the midbrain. It is extremely difficult to localize lesions in this region of the brain, and so far only 2 partially satisfactory results have been obtained. In the example given the left nuclei were completely destroyed:

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[1] Moore, PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi 48.

Time	Temp. ° C.	Tonus symptoms
9:30 a. m.	39.5	
10:00 a. m.	Injection of chromic acid	
10:10 a. m.	36.4	Relaxed, nystagmus.
10:20 a. m.	35.6	Very relaxed.
10:50 a. m.	34.4	Cannot stand.
11:15 a. m.	33.9	
11:45 a. m.	33.0	Marked hypotonia.
12:15 p. m.	32.7	
12:45 p. m.	32.3	Hypotonia.
1:15 p. m.	32.5	
2:30 p. m.	33.0	
5:00 p. m.	34.2	Slight increase in tonus.
9:00 p. m.	34.2	

The rate of the change in temperature and in tonus very exactly corresponds to that obtained by many midbrain lesions and it is possible that if both the right and left nuclei are destroyed the profound effects of the extreme midbrain cases will be found. The parallelism is sufficiently exact to make plausible the hypothesis that the hypothermia following the midbrain lesions is due to injury of the fibers from the vestibular nuclei which run in the posterior longitudinal bundle and not to dysfunction of a hypothetical heat center. Many more cases are needed, however, before a definite conclusion can be drawn.

Missouri Branch.

St. Louis University Medical School, October 24, 1928.

4164

A Method for Determination of Volatile Fatty Acids in Stools.

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From the Department of Internal Medicine, Washington University School of Medicine, St. Louis, Mo.

In a previous report[1] it was shown that the volatile fatty acids, formic, acetid and butyric, could be steam distilled from a concentrated solution of magnesium sulphate at rates of 60 to 100% faster than from watery solutions. We wish to report here a method for the precipitation of stools and the application of the rapid method of steam distillation to the filtrates obtained.

Stools were collected in a volume of 10% sodium hydrate equal to that of the stool. The alkali stops fermentation almost completely, deodorizing the stool, and favors emulsification. 100 gms. of alkali stool mixture is neutralized with strong sulphuric acid and 175 to 200 cc. of a 10% mercuric chloride solution added. This amount of mercuric chloride is soluble in 1.5% hydrochloric acid. The mercury is precipitated with solid calcium hydrate, which is added until the stool is strongly alkaline to litmus. The volume is made up to 500 cc. which is centrifuged in 100 cc. centrifuge tubes. Two 100 cc. portions of supernatant fluid are measured off and made acid to litmus with sulphuric acid. Excess mercury is precipitated with hydrogen sulphide, the excess being blown off with a current of air. After filtering 100 cc., portions of filtrate are distilled in the manner reported.

These filtrates from stools are almost nitrogen free (95% being removed) and free from higher insoluble fatty acids and sugar. On distilling the stool filtrates the rates of distillation obtained closely resemble those obtained from mixtures of pure volatile fatty acids. When formic, acetic, propionic or butyric acids are added to stools they may be recovered to within 5 to 10%. Duplicate determinations usually agree to within 5%. From the observed rates of distillation, it is concluded that the principal volatile fatty acids in the stools are acetic and butyric.

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[1] Olmsted, W. H., and Whitaker, W., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 897.

4165

Changes in Thyroid Gland Following Feeding of KI and Anterior Lobe of Pituitary.

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In studies on compensatory hypertrophy following partial thyroidectomy, Loeb[1] has shown that feeding potassium iodide stimulates regenerative activity while anterior pituitary substance prevents hypertrophy and produces a hard colloid and flattening of the acinar cells. The change induced by potassium iodide in the normal intact thyroid has recently also been studied and a method devised for determining, in a quantitative manner, the proliferative activity by estimating the total number of mitoses in the thyroid gland at any given time [2],[3].

These experiments were undertaken to study the effect of combined feeding of these 2 substances and to determine whether or not the inhibiting action of pituitary would render the thyroid unresponsive to the stimulating influence of potassium iodide. If complete physiological neutralization of the stimulating influence of potassium iodide and of the depressing action of pituitary should take place in animals fed with these substances, the thyroid should remain unaltered, but if one were dominant the degree of its influence could be estimated by the mitotic activity of the gland.

Three series of experiments were carried out using in all 45 guinea pigs. They were divided into 4 groups (1) animals fed KI, each receiving 0.05 gm. daily; (2) those fed one 5 grain tablet of anterior pituitary substance (Armour & Co.) every day; (3) those on combined feeding receiving daily both 5 grain pituitary tablet and the 0.05 gm. pill of KI. (4) Control animals. One series consisting of 5 animals in each group were fed for 20 days; the other 2 series were both fed for 15 days.

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[1] Loeb, Leo, *Am. J. Path.*, 1926, ii, 19; *J. Med. Res.*, 1920, lxiv, 557; *J. Med. Res.*, 1920, lxii, 77.

[2] Gray, S. H., and Loeb, Leo., *Am. J. Path.*, 1928, iv, 257.

[3] Rabinovitch, Jacob, Proc. Soc. Exp. Biol. and Med., 1928, xxv, 812; *Am. J. Path.*, 1928, iv, No. 6.

Serial sections were made from all glands and the number of mitoses in each estimated by counting those in every tenth section. In studying the sections the size of the acini, the consistency of the colloid and the character of the epithelial cells, in the different groups, were compared.

I. *The number of mitoses.* The average number of mitoses per gland for all the animals fed KI was 1208; for the pituitary fed group, the average was 62; the group on combined feeding of KI and pituitary substance showed an average of 150; and the control group averaged 170 mitoses per gland.

II. *Histological character of the thyroids. KI fed animals.* The acini are larger than in controls. The epithelial cells are slightly hypertrophied in the 15 days series and at this time, especially, they show many mitotic figures. The colloid is often softer and stains less intensely than in controls. There are many phagocytes within the colloid in some of the acini especially in the 20 day series.

Anterior pituitary fed animals. There is a striking contrast in structure between this group and the controls. The acini are smaller and more crowded together. They are filled with a very dense non-vacuolar colloid completely filling the acini and compressing the lining epithelium into a slender strand of flattened cells.

Combined feeding with KI and pituitary. The acini are slightly larger than the pituitary fed animals and are not so crowded together. The colloid is solid but not as dense as in the preceding groups and extends to, but does not compress the cells which are almost as tall as in normal glands.

These thyroids stand midway between the appearance of those from animals fed pituitary and from controls. They resemble the latter in that the acini are of about normal size and are separated by thin strands of connective tissue and in that the epithelium is almost of normal height. The mitotic index is also within normal limits. The hardness of the colloid and the manner in which it completely fills the acini without the occurrence of vacuoles suggest the influence of pituitary substance. It seems likely that the relative dosage of these 2 substances could be so balanced that the thyroids would remain unchanged and show the influence of neither.

4166

Combinations of Various Metals with Urease of Amoebocytes of Limulus or with its Coenzyme.

LEO LOEB AND I. LORBERBLATT.

From the Department of Pathology, Washington University School of Medicine, St. Louis, and the Marine Biological Laboratory, Woods Hole, Mass.

In former papers Loeb and Bodansky[1] have shown that if amoebocyte tissue of Limulus is extracted with various salts, the activity of urease in the extract differs very much in accordance with the character of the metal present in the salt. Furthermore, Loeb, Lorberblatt and Field[2] found that after extraction with NaCl a subsequent addition of $CaCl_2$ is without benefit, while after extraction with $MgCl_2$ subsequent addition of $CaCl_2$ increases markedly the efficiency of the extract.

In continuing these investigations we found that after extraction with certain salts in which the character of the anion is the same but which differ in respect to the kation, the efficiency coefficients of the various urease preparations differ very much from each other; we found that by adding a second salt to the extract made with a first salt the urease preparation can, under certain conditions, be made to assume the efficiency coefficient characteristic of the second salt and the readiness with which this transformation can be accomplished may be represented in definite curves, the abscissa of which indicates the time at which the second salt was added and the ordinate the amount of urease transformed into the new urease preparation. These curves are specific for each combination used. Thus, if we add NaCl to $CaCl_2$ extract one hour after the beginning of the extraction, almost one-half the $CaCl_2$ effect can be replaced by the NaCl effect; but during the next 4 hours the $CaCl_2$ effect gradually increases in strength and at last has become so strong that it remains unaffected by addition of NaCl. In the urease preparation obtained by extraction with NaCl, only within the first 30 minutes after the beginning of extraction can the $CaCl_2$ effect replace to a slight extent the NaCl effect. After completion of extraction (2 hours) the effect of addition of Ca has become nil. Thus each preparation has a specific time curve. These facts can best be explained if we assume that definite metal-urease or metal-coenzyme combinations form, which in some curves are at first of a relatively loose character, but which become more firm in the course of the next few hours; the rapidity with which this change takes place varies in the case of different metals. In this connection we have not only to consider the primary metal combination but also the second metal, which is meant to replace the first one. Thus the Na combination becomes a very firm one soon after the beginning of extraction with a NaCl solution, while the Ca combination is at first relatively loose and the Ca in this combination can much more readily be replaced by Na, but, as stated, after having been in contact with the urease for 3 hours after completion of extraction, it can no longer be replaced by Na. The Mg combination behaves approximately like the Ca combination. The Mn combination, on the other hand, becomes at once so firm that from the very beginning the Mn cannot be dissociated from the urease (or coenzyme) through the addition of other salts as far as we have tested the latter in our experiments. Conversely Mn can readily replace Ca and Mg.

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[1] Loeb, L., and Bodansky, O., *J. Biol. Chem.*, 1926, lxvii, 79; *J. Biol. Chem.*, 1927, lxxii, 415.

[2] Loeb, L., Lorberblatt, I., and Field, M. E., *J. Biol. Chem.*, 1928, lxxviii, 417.

The fact that these metal-urease or coenzyme combinations became fixed after a certain time, which varies considerably in the case of different metals, makes it seem improbable that the metals are combined with the urease or coenzyme in the form of readily dissociable ion combinations; it rather appears as if combinations of a different nature develop under these conditions.

Whether the metals in these combinations are bound to the urease or to a hypothetical coenzyme cannot be answered definitely at the present time, but the fact that Ca extracts, in which the urease efficiency has been weakened through heating, do not show an activating effect on NaCl extracts or on urease in blood serum, as well as certain other facts, make it more probable that we have to deal with metal-urease combinations. However, we intend to study this problem further especially by means of diffusion experiments.

Previously we have found that urease occurs in various kinds of cells and also in blood serum of Limulus, but apparently not in other arthropods. There is every reason for assuming that the urease present for instance in the blood serum and the blood cells of Limulus is of the same kind. It was of interest to determine whether the urease in all tissues of Limulus shows the same specific relations to salts which we observed in the case of urease extracted from the amoebocyte tissue. We found this not to be the case; neither does lipase extracted from amoebocyte tissue of Limulus show comparable reactions. We must then conclude that the metal urease combinations, which we observed, are specific for the particular constellation in which urease occurs in amoebocyte tissue of Limulus. These conditions can be altered, if we extract with certain salts, one constituent of which undergoes at once a very firm combination with urease (coenzyme), and they are also modified if we extract with blood serum which contains protein. It might be suggested therefore that, under usual circumstances, enzymes are in stable union, either with constituents of salts or with proteins, which make it impossible for them to form readily other combinations comparable to those into which the urease of amoebocyte tissue of Limulus or its enzyme can enter.

4167

Fate of Skin Bacteria after Autogenous and Heterogenous Transplantation of Skin into Subcutaneous Tissue.*

L. ERMATINGER, F. B. QUEEN and R. F. PARKER
(Introduced by Leo Loeb)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

In comparing auto-, homoio- and heterotransplants of various tissues Loeb observed, in general, a greater number of polymorphonuclear leucocytes around or in the transplanted tissues in the last type of transplantation than in the 2 former. The question arose whether the appearance of these leucocytes was due to the presence of bacteria in heterotransplants or whether the leucocytes were attracted directly by the heterotoxins. These observations suggested the following investigations in which we wished to determine (1) what the fate is of bacteria normally present on the skin after transplantation into the subcutaneous tissue, (2) whether in different species the fate of the bacteria after subcutaneous transplantation differs, and (3) whether there is a difference in the fate of bacteria adherent to the skin in case of autogenous and heterogenous transplantation.

We transplanted pieces of ear skin of approximately the same size into pockets of the subcutaneous tissue. Before transplantation the hair of the skin to be used was clipped, but otherwise the skin was left unchanged. In different experiments the pieces were left in the subcutaneous pockets 1, 2 or 3 days before they were removed, with precautions which excluded bacterial contamination. We then determined the changes in the total number of bacteria in the transplants and the changes in the various types of bacteria found in the skin. As controls we made determinations of the bacterial flora of the normal ear skin of the animal that served as donor. The methods for the determination of the number of bacteria adherent to the surface of a certain area of skin, do not permit a quantitatively accurate counting of all the microorganisms present; but, inasmuch as the same methods were employed in all cases, approximate results are obtained which allow certain conclusions to be drawn. The animals used were guinea-pigs, rats and rabbits. In each species we studied autotransplants of skin. We also studied heterotransplants from guinea-pigs to rat, from rat to guinea-pig, from rabbit to guinea-pig and from guinea-pig to rabbit.

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* We wish to express our appreciation to the Department of Bacteriology of Washington University School of Medicine and in particular to Dr. Upton for courtesies received in the course of these investigations.

One hundred and four animals were used in these experiments which served either as donors or hosts or as both donor and host of the skin transplants (26 rabbits, 52 guinea-pigs and 26 rats). In some animals only one piece of ear was transplanted, into others 2 or even 3 pieces were transplanted. The number of skin pieces transplanted and examined bacteriologically was as follows:

I. *Autogenous transplantation* in the rabbit: 27 pieces; in the guinea-pig: 33 pieces; in the rat: 15 pieces.

II. *Heterogenous transplantation:* Rabbit to guinea-pig: 8 pieces; guinea-pig to rabbit: 6 pieces; rat to guinea-pig: 26 pieces; guinea-pig to rat: 19 pieces. In addition, control pieces of skin, of approximately the same size as the transplants were examined in each experiment.

We can at the present time draw the following conclusions: 1. There was a progressive decrease in the number of bacteria found in the transplants on successive days. The large majority of the microorganisms was destroyed within the first 24 hours although in only 1 or 2 instances were entirely sterile plates found after one day. The destruction was still more complete after 48 hours, when nearly 25% of the plates were sterile. After 3 days, sterile plates were found in the majority (62%) of cases, and, in the rest, the number of colonies in the plates was very small. 2. In most instances, staphylococcus was the only survivor after 24 hours, and after 48 hours all the other organisms were destroyed except staphylococcus.

We record provisionally the following observations which, we believe, need further corroboration. 3. We found some deviation from this typical curve, representing the destruction of bacteria, during the summer months: During the very hot season we found in a number of cases instead of a decrease, a considerable increase in the number of bacteria in the transplants within the first 3 days, in the rat as well as in the guinea-pig. Furthermore, the count of the bacteria living on the normal skin seemed to be higher during the hot weather of the summer than during other seasons. Except for the effect of great heat ordinary variations in temperature did not seem to affect the fate of the bacteria. 4. If we compare the readiness with which the bacteria of the skin are destroyed after subcutaneous transplantation in these 3 types of animals we find the rabbit to be the most effective in this respect, and the rat to be almost as much so, while the guinea-pig destroyed the microorganisms somewhat less actively than the other species. However, on the whole the curve representing the diminution of bacteria on successive days was similar in these 3 species. 5. It seemed that the destruction of bacteria proceeded more actively after hetero- than after autotransplantation of the skin. We are continuing these experiments.

4168

Erythrocyte Sedimentation Rate of Citrated Blood and Distribution of Plasma Proteins.

ROBERT JONES (Introduced by M. S. Fleisher)

From the Department of Bacteriology and Hygiene, St. Louis University.

An attempt was made to determine the relationship existing between an increased erythrocyte-sedimentation-rate (ESR) and the quantitative distribution of plasma proteins. In the venous blood of the dog it was observed that a marked increase in the ESR occurs promptly upon the establishment of a sterile abscess produced by subcutaneous administration of turpentine, which is characterized by endogenous tissue absorption. The ESR rapidly returns toward normal upon absorption, or surgical drainage of the exudate. Significant changes in the quantitative distribution of the protein fractions of the plasma were observed, incident to this type of systematic stimulation and appear to have a correlation with the various phases of the rather acute inflammatory process, as is outlined in Table I.

TABLE I.
TURPENTINE ABSCESS IN DOGS.
Viscosity and Protein Content of Plasma with Relation to the ESR.

	Date—January	Plasma Protein %	Fibrin %	Protein Quot.	Plasma Viscosity	ESR 2 hours	
Dog No. 1	16	8.41	0.40	1.4	1.670	.05	Normal
	16	—	—	—	—	—	Turpentine subcutem 2 cc.
	20	6.58	0.28	2.1	1.914	.14	Large indurated mass palpable under skin
	23	8.14	0.80	1.1	2.368	.55	Marked softening
	25	7.50	0.50	3.4	2.000	.46	Drainage of abscess
	28	6.40	0.34	1.6	1.726	.38	Healing
Dog No. 2	20	5.77	0.32	1.6	1.708	.17	Normal
	20	—	—	—	—	—	Turpentine subcutem 2 cc.
	24	7.60	0.73	1.3	2.277	.57	Softening of inflammatory mass
	27	6.24	0.65	2.2	1.954	.35	Marked absorption
	30	6.80	0.41	1.4	1.786	.28	Absorption nearly complete

Following the establishment of a definite inflammatory process in the dog, an increase in plasma viscosity, presumably incident to the lesion, is associated with a somewhat increased amount of total protein, with a significant increase in the amount of circulating fibrinogen and a very much accelerated velocity of sedimentation of the erythrocytes. These changes in the amount of fibrinogen and in the ESR are of considerable magnitude and correlate closely in time of appearance and extent with the existing pathologic state experimentally induced. Changes observed in the value of the protein-quotient are not wide, fluctuate somewhat irregularly and barely suggest an increasing ratio of globulin to albumin, with progression of the induced lesion.

In addition, in an extended study of the arterial blood of normal rabbits, and, of rabbits subjected to stimuli afforded by sterile abscess and by tuberculosis of visceral organs, this relationship of an increased ESR, increase in fibrinogen and increase in plasma viscosity, was observed with regularity. Likewise in the examination of venous blood of humans with acute infections these changes in the properties of blood and of plasma were co-existent. From the data available, it is not to be considered that an increase in the velocity of sedimentation of red blood cells is dependent upon an increase in the amount of fibrinogen in the plasma, but that under these types of experimental stimulation these changes were observed to be concomitant.

4169

Cause of Death Following Intravenous Injection of Ox and Dog Serum into Rabbits.

JACOB RABINOVITCH. (Introduced by Leo Loeb.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

Marked divergencies occur[1, 2, 3, 4, 5, 6, 7, 8] concerning the mode of action of foreign sera in causing death after intravenous injection. We repeated and extended in various directions the investigations of Loeb and his collaborators, and of Kusama. Our results are as follows:

I. *Injection of ox serum.* When we injected a lethal dose of ox serum into the ear vein of a rabbit, we found in the lungs removed immediately after the death of the animal, very extensive occlusion of the capillaries as well as of the larger vessels by thrombi. In the capillaries these thrombi appear to consist of agglutinated red blood cells only; whereas in the larger vessels there was also an admixture of white blood cells and possibly of platelets; no fibrin could be made out anywhere. All the other organs examined microscopically showed merely congestion. The injection of a sub-lethal dose of ox serum caused only partial occlusion of the pulmonary vessels by such thrombi and the animal therefore survived. When heparin was injected in quantities sufficient to prevent coagulation of the blood together with a lethal dose of ox serum, it did not prolong the life or prevent the death of the animal. Also the microscopic picture of the various organs, remained the same as after injection of the ox serum alone.

II. *Injection of dog serum.* After injection of a lethal dose of dog serum, microscopic examination of the lungs also showed massive occlusion of the capillaries and larger vessels by thrombi. In the capillaries the thrombi appeared to consist of massed-together, pale red blood cells, without any fibrin being visible; in the larger vessels however, fibrin could be definitely seen in the

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[1] Landois, *Die Transfusion des Blutes.* Leipzig, 1875.

[2] Flexner, Simon. *J. Med. Res.*, 1902, viii, 316.

[3] Loeb, Leo, Strickler, A. and Tuttle, L. *Virchow's Archives*, 201, 5, 1910.

[4] Kusama, S. *Ziegler's Beitr.*, 1913, v, 55.

[5] Airila, Y. *Skand. Arch. fur Physiol.*, 1914, xxxi, 388.

[6] Coca, A. *J. Immun.*, 1919, iv, 219.

[7] Drinker, C. K., and Bronfenbrenner, J., *J. Immun.*, 1924, ix, 5.

[8] Friedberger, E., and Seidenberg, S., *Z. f. Immun. Forsch.*, 1927, li, 216.

thrombi. The finding of fibrin in the larger vessels as well as the action of heparin in combination with dog serum, to be referred to shortly, suggests very strongly however, that the thrombi formed in the capillaries also contained fibrin, which was however, too fine to be recognized microscopically. When heparin was injected in combination with a lethal dose of dog serum, the life of the animal was prolonged for a long time and in some cases death was prevented altogether. Microscopically no thrombi were seen in the lungs of the animals that thus survived. Experiments *in vitro* showed that heparin in addition to its anticoagulative action is capable of preventing hemolysis of the rabbit's red blood cells normally caused by dog serum. Thus heparin prevents the formation of fibrin thrombi in this case in a double way namely, (1) by preventing coagulation and (2) by diminishing the setting free of the tissue coagulin (thrombokinase) from the erythrocytes.

Following the injection of a lethal dose of either ox or dog serum there resulted a very marked reduction of all the cellular elements of the rabbit's blood. In sublethal doses, we found that ox serum produced only a temporary reduction of the cellular elements of the blood which in the course of 24 hours tended to return to normal. Dog serum, on the other hand, produced a noticeable reduction in the number of red blood cells only, and this reduction was still present after 24 hours because of the hemolysis caused by injection of dog serum. Heparin which did not influence the effect of ox serum on rabbit's blood cells, prevented the reduction of the blood cells caused by dog serum, owing to its antihemolytic effect. After injection of ox serum the loosely agglutinated cells which are held back in various organs are freed in the course of 24 hours and are again free to circulate in the vessels.

It follows from our experiments that both ox and dog serum when injected in lethal doses intravenously into rabbits cause the death of these animals through the formation of thrombi which in the case of ox serum consist of agglutinated erythrocytes and in the case of dog serum of fibrin thrombi. While this conclusion seems definite in the case of dog serum, there remained in the case of ox serum still the possibility that constriction of the pulmonary vessels may after all be the real cause of death and that the presence of agglutination thrombi is merely simulated. We attempted therefore, to demonstrate directly the occlusion of the pulmonary vessels by examining on glass slides thin slices of lung removed rapidly before secondary changes could take place, from rabbits following injection of lethal doses of ox serum. By exerting gentle pressure, we could squeeze out from the vessels numerous thrombi consisting of agglutinated red blood cells which were readily seen under the microscope.

Our results confirm and extend thus the results obtained by Loeb, Strickler and Tuttle and they are contrary to the conclusions of Kusama. There remains of course the possibility that in case of agglutinative sera a constriction of pulmonary vessels may play a certain role in addition to the formation of agglutination thrombi; but at best the significance of the latter factor could only be of a subsidiary character.

New York Meeting.

Rockefeller Institute, November 21, 1928.

4170

A Mechanism Maintaining the Hyperglycemia of Diabetes.

H. E. HIMWICH, W. H. CHAMBERS, Y. D. KOSKOFF, L. H. NAHUM AND M. A. ADAMS.

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In a previous report[1] it was shown that resting muscle of normal dogs may liberate lactic acid into the blood stream. The lactic acid may thus reach the liver where it is removed and converted to carbohydrate. It may be retained in the liver as glycogen or leave the liver, appearing in the blood as glucose. In the present study on diabetic dogs it was found that this lactic acid-glucose cycle involving muscle and liver was one of the mechanisms for maintaining hyperglycemia after the glycogen stores of the liver were reduced by fasting.

Fourteen large female dogs were fasted for 3 days or more and were then depancreatized under aseptic precautions. When the respiratory quotient and the D/N ratio indicated that the dogs had become completely diabetic, they were put under amytal anesthesia, the femoral vessels were exposed and the abdomen was opened by a longitudinal incision. Samples of blood were drawn practically simultaneously from the femoral artery, femoral vein, portal vein and hepatic vein. These blood samples were analyzed for glucose and lactic acid.

The results may be presented as 3 links in a chain of evidence: First as seen in Table I, both during rest and exercise there is greater concentration of lactic acid in the blood of the femoral vein than in that of the femoral artery. Taking differences of 5 mg. % or more as significant, this occurred in 13 of 25 observations. Only once did the muscles take out lactic acid from the blood.

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[1] Himwich, H. E., Koskoff, Y. D., Nahum, L. H., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 347.

TABLE I.

Lactic Acid of the Femoral Artery and the Femoral Vein.

	Lactic Acid mg. %	
	Femoral Artery	Femoral Vein
Resting blood	29	30
84 minutes of work	31	38
106 minutes of work	38	49

TABLE II.

Lactic Acid of the Blood Entering and Leaving the Liver.

		Lactic Acid mg. %	
	Femoral Artery	Portal Vein	Hepatic Vein
Resting blood	58	60	46
12 minutes of work	79	73	61
65 minutes of work	75	80	61

TABLE III.

Glucose of the Blood Entering and Leaving the Liver.

		Glucose mg. %	
	Femoral Artery	Portal Vein	Hepatic Vein
Resting blood	345	350	380
12 minutes of work	380	360	385
65 minutes of work	380	390	435

As shown in Table II, the second link in the chain of evidence, is the removal of lactic acid by the liver. In 16 of 22 observations the liver removed lactic acid from the blood and in but one instance did it add lactic acid.

The last link in the evidence (Table III) is the liberation of glucose by the liver. Despite the hyperglycemia, in 18 of 22 observations the liver added glucose to the blood passing through it. Only twice did the liver remove glucose from the blood stream.

In most instances the liver added more glucose to the blood than could be accounted for by the lactic acid entering that organ. This additional glucose is probably produced in the heightened processes of protein metabolism. However, our chief point is the fact that the liver forms carbohydrate from the lactic acid liberated by muscle. Since in diabetes this carbohydrate is not retained by the liver as glycogen it necessarily appears in the blood as glucose. This mechanism is therefore an important factor in maintaining the hyperglycemia of diabetes.

4171

Fate of Blood Sugar During Glycolysis.

ICHIRO KATAYAMA AND TERESA GARCIA.

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A review of the literature on glycolysis warrants the conclusion that the sugar of shed blood diminishes on standing, independent of bacterial contamination. With respect to the fate of the lost sugar, several papers have been reported in the literature, deducing somewhat similar conclusions, that a portion of the lost sugar is converted into lactic acid. However, there remained the uncertainty as to the quantitative relation of the sugar lost to the amount of lactic acid produced.

In order to clear up this point, we carefully studied the blood glycolysis *in vitro* from the point of view of the simultaneous changes taking place in the glucose and lactic acid contents of the blood.

The blood was drawn in all instances from the veins under aseptic conditions and maintained at 38° C. free from bacterial contamination. Analyses of the blood were made immediately and after 3, 6 and 24 hours. Potassium oxalate was employed as an anti-coagulant for the whole blood. The amount of the potassium oxalate used was roughly 0.06% of the blood. The specimen was cultured at the end of 24 hours and thus bacterial contamination was excluded.

Analytical Procedures: Brehme and Brahdy[1] have recently described a comparatively simple and accurate method for the estimation of the lactic acid of blood. Hence we employed their method for the determination of lactic acid in the blood. The sugar of blood we determined by the Shaffer-Hartmann method.[2]

Discussion of Results: The results are presented in Table I. The findings for 10 subjects showed that the *in vitro* process of glycolysis, the sugar gradually decreases with a parallel rise in the lactic acid. All instances except one (subject 2) showed that the difference between the amount of disappeared sugar and the increased lactic acid during 24 hours lies within 12 mg. In subject 2 the difference between these 2 substances was 20.5 mg.

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[1] Brehme, T., and Brahdy, B., *Biochem. Z.*, 1926, clxxv, 348.

[2] Shaffer, P. A., and Hartmann, A. F., *J. Biol. Chem.*, 1921, xlv, 365.

TABLE I.
Glycolysis and Production of Lactic Acid of Blood.

Case	Age	Sex	Test Hour	Sugar	Lactic Acid	Lost Sugar	Gained Lactic Acid	Difference Sugar-Lactic Acid
				mg. per 100 cc.		mg. per 100 cc.		
1	35	M	At once	92	24.6			
			3 hrs.	74	46.0	18	21.4	— 3.4
			6 hrs.	74	59.2	18	35.6	—17.6
			24 hrs.	18	107.7	74	83.1	— 9.1
2	28	F	At once	109	22.7			
			3 hrs.	92	47.3	17	24.6	— 7.6
			6 hrs.	66	59.2	43	37.5	+ 5.5
			24 hrs.	18	93.2	91	70.5	+20.5
3	20	M	At once	99	22.1			
			3 hrs.	76	40.3	23	18.2	+ 4.8
			6 hrs.	66	55.4	33	33.3	— 0.3
			24 hrs.	18	91.9	81	69.8	+11.2
4	25	M	At once	122	18.3			
			3 hrs.	89	39.7	33	21.4	+11.6
			6 hrs.	66	55.4	56	37.1	+18.9
			24 hrs.	25	105.8	97	87.5	+ 9.5
5	32	M	At once	154	19.5			
			3 hrs.	129	32.8	25	13.3	+11.7
			6 hrs.	114	46.0	40	26.5	+13.5
			24 hrs.	99	82.5	55	63.0	— 8.0
6	41	F	At once	119	18.3			
			3 hrs.	104	30.9	15	12.6	+ 2.4
			6 hrs.	87	43.5	32	25.2	+ 6.8
			24 hrs.	39	95.8	80	77.5	+ 2.5
7	36	M	At once	114	16.4			
			3 hrs.	92	32.8	22	16.4	+ 5.6
			6 hrs.	76	41.6	38	25.2	+12.8
			24 hrs.	28	93.2	86	76.8	+ 9.2
8	21	F	At once	111	15.8			
			3 hrs.	89	30.9	22	15.1	+ 6.9
			24 hrs.	39	83.1	72	67.3	+ 4.7
9	13	M	At once	95	24.6			
			3 hrs.	66	51.0	29	26.4	+ 2.6
			24 hrs.	14	105.2	81	80.6	+ 0.4
10	12	M	At once	92	22.7			
			3 hrs.	66	47.9	26	25.2	+ 0.8
			6 hrs.	36	62.4	56	39.7	+16.3
			24 hrs.	5	104.7	87	82.0	+ 5.0

Levene and Meyer[3] have shown that when leucocytes were suspended in Henderson's phosphate solutions containing glucose, a portion of the glucose, as such, disappeared with the production of lactic acid. Evans[4] has observed a fall in the carbon dioxide capacity of shed blood which, he believes, is due to a conversion of glucose into lactic acid as a result of glycolysis. Kraske[5] in his studies on 7 human subjects reported a quantitative conversion of the lost blood sugar into lactic acid during an incubation of 2 hours. Kondo,[6] however, in his experiments on glycolysis in dog's blood during a similar period of incubation found that the increase in lactic acid was much less than the amount of sugar lost. Morgulis and Berkus[7] have reported that the disappearance of the sugar proceeds parallel with a formation of lactic acid. Their figures, however, do not demonstrate this parallelism. Katayama[8] failed to find a production of carbon dioxide as a result of glycolysis.

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[3] Levene, P. A., and Meyer, G. M., *J. Biol. Chem.*, 1912, xi, 361.

[4] Evans, C. L., *J. Physiol.*, 1922, lvi, 146.

[5] Kraske, B., *Biochem. Z.*, 1912, xlv, 81.

None of the observers in the literature did demonstrate substances other than lactic acid produced as a result of glycolysis. In our experiments we confirmed the quantitative recovery of lactic acid from the blood sugar during glycolysis.

Summary: The sugar of shed blood gradually decreases on standing without bacterial contamination. The disappearance of the blood sugar is accounted for by the formation of a corresponding amount of lactic acid.

4172

Reversible Changes in Living Protoplasm.

W. J. V. OSTERHOUT AND E. S. HARRIS.

From the Laboratories of The Rockefeller Institute for Medical Research.

It has been shown[1] that when a cell of *Nitella* is cut a wave of some sort, which we may for convenience call a death wave, passes rapidly along the cell and that at each spot it reaches a characteristic death process is brought about. In dilute solutions (*e. g.* 0.001 M KCl) this consists of a sudden change whereby the protoplasm becomes more negative after which its potential difference approaches zero.

These alterations are irreversible but similar changes of a reversible character may occur spontaneously or may be induced by a variety of reagents (both organic and inorganic). After the removal of the reagents the changes often cease at once or after some minutes. Very often these changes are rhythmical and appear to travel along the cell, sometimes at a high rate of speed.

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[6] Kondo, K., *Biochem. Z.*, 1912, xlv, 88.

[7] Morgulis, S., and Berkus, O., *J. Biol. Chem.*, 1925, lxv, 1.

[8] Katayama, I., *J. Lab. and Clin. Med.*, 1926, xii, 239.

[1] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928-29, xii, 167.

During the past 5 years a large number of such cases have been recorded photographically by means of the technique previously described.[2] These are being studied with a view to correlating all the phenomena since, to arrive at a theory of living matter, it is necessary to have accurate observations of its fluctuations under normal conditions. It would seem that such alterations might be due to changes in the permeability of the surface or in resistance elsewhere. Such changes in the surface might be essentially structural (*e. g.*, due to the temporary formation of openings in certain layers) or chemical in nature.

4173

Predicting Penetration of Dyes into Living Cells by Means of an Artificial System.

MARIAN IRWIN.

From the Laboratories of The Rockefeller Institute.

The penetration of dyes into the vacuoles of living cells of *Nitella* and *Valonia* proceeds as though the cell consisted of a non-aqueous layer lying between the external aqueous dye solution and the internal aqueous sap of the vacuole. On this basis it should be possible to predict the relative rate of penetration of dyes into the vacuoles of living cells if we know their rate of diffusion into the "vacuole" of an artificial system representing the 3 phases just mentioned.

To test this an artificial system was constructed consisting of a horizontal glass tube with 3 vertical arms. (1) To the left arm is added the dye solution (identical with the one in which cells are placed, *i. e.* sea water for *Valonia* and buffer solution for *Nitella*); (2) to the central arm is added chloroform (representing the non-aqueous layer of the living cell) until it fills the horizontal portions and the lower part of each upright tube. (3) Upon the chloroform in the right arm is poured some of the sap, artificial or freshly extracted from the living vacuole of *Valonia* or *Nitella* as the case may be (this will be called the artificial "vacuole"). Each one of these 3 phases is stirred by a separate glass stirrer entering through the corresponding upright arm. All the stirrers revolve at a uniform rate. Dye which penetrates into the chloroform at the phase boundary is rapidly distributed uniformly throughout the mass of chloroform. The rate of passage of the dye from the dye solution through chloroform into the sap (the artificial "vacuole") is chiefly determined by the rate of diffusion at each phase boundary, which in turn is partly determined by the partition coefficient of the dye between the non-aqueous and the aqueous phase.

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[2] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927-28, xi, 391, 673.

The concentration of dye in the left arm was kept as constant as possible. The system was stirred for one hour after which the concentration of dye was colorimetrically determined in the dye solution (left arm) and in the sap (right arm).

The corresponding rate of penetration of dye into the vacuoles of cells in normal condition was determined as previously described.

A striking similarity exists between this artificial system and a living cell in respect to the control of the rate of penetration. (1) Cresyl blue, azure B, basic fuchsin, toluidine blue, new methylene blue, methylene green, penetrate the living and the artificial "vacuoles" more readily at pH 9.5 than at pH 5.5. This is also true of *Nitella* and *Valonia*. (2) Trypan blue, trypan red, Congo red, alizarin S, methyl blue, and Bordeaux red penetrate neither the living vacuole nor the artificial "vacuole". With acid fuchsin a trace is found in the artificial vacuole and none in *Nitella* and *Valonia*. (3) *a*. With crystal violet and methyl violet penetration was very slow in all cases. The rate was not appreciably altered with a change in the pH value. *b*. With methyl green a trace of green dye penetrated into the artificial "vacuole" and into the living vacuoles of *Valonia* and *Nitella*. The methyl green solution was green in color but a violet dye was readily absorbed by chloroform. Spectrophotometric measurement showed that this violet dye was crystal violet while the green dye was methyl green. *c*. With thionine in sea water a trace of a pale violet pink dye penetrated the artificial "vacuole" containing *Valonia* sap and into the living vacuole of *Valonia*; with thionine in buffer solution a trace of pale violet dye penetrated into the artificial "vacuole" containing artificial *Nitella* sap and into the living vacuole of *Nitella*. More dye penetrated at pH 9.5 than at pH 5.5. The thionine solution appeared violet but the dye absorbed from it by chloroform gave a pinkish orange color to the chloroform. Both these dyes were found to give absorption curves characteristic of thionine. The amount absorbed by the sap was too small to permit spectrophotometric measurement.

Overton's theory,[1] which involves only one partition coefficient and which states that only substances soluble in lipoid can penetrate living cells might explain the penetration of dyes in the first 2 groups (with the exception of acid fuchsin and alizarin S). But the behavior of the third group cannot be explained by his theory. For example, crystal violet and methyl violet are readily soluble in chloroform and yet they penetrate the vacuole very slowly.

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[1] Overton, E., *Jahrb. wissensch. Bot.*, 1900, xxxiv, 669.

On the other hand the behavior of all the groups may be explained by the theory[2] which postulates 2 or more partition coefficients and which is illustrated by the artificial system.

4174

Is Respiratory Anaphylaxis (Asthma) the Result of a Local or General Sensitization?*

BRET RATNER AND HELEN LEE GRUEHL.

From the Departments of Immunology and Pediatrics, University and Bellevue Hospital Medical College, New York University.

When we made our investigations[1] on respiratory anaphylaxis (asthma) we did not know whether the sensitization brought about by the inhalation of a dry antigenic dust was general or purely local in character.

If sensitization and shock are brought about through inhalation, and, anaphylactic death demonstrated by a subsequent intravenous injection, it is plausible to assume that this sensitization might be relegated exclusively to the respiratory tract. However we have no definite evidence to support either the local or general character of this particular form of hypersensitiveness.

More recently[2] we showed that a pregnant guinea pig who had demonstrated respiratory anaphylaxis throughout pregnancy actually transmitted similar hypersensitiveness to her offspring *in utero*. This points to the assumption that inhaled antigens do circulate in the blood of the sensitized animal. In order to show further proof for this fact we have carried out the following experiments.

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[2] Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 127.

* This work is being carried on under "The Crane Research Fund for the Study of Allergic Diseases in Children."

[1] Ratner, B., Jackson, H. C., and Gruehl, H. L., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 16; *ibid*, p. 17; *Am. J. Dis. Child.*, 1927, xxxiv, 23.

[2] Ratner, B., and Gruehl, H. L., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 8.

Young virgin guinea pigs were sensitized by inhalation to dry horse dander dust. After a suitable incubation period, a laparotomy was performed under ether anesthesia and the uterine strip was removed. This strip was suspended in an oxygenated bath of Locke's solution and the Dale experiment was carried out in the usual manner, using an extract of horse dander as antigen.[1] After the excision of the uterine strip, the abdomen was sewed up and the animal allowed to completely recover from her anesthesia. When the animal was apparently normal again it was given an intravenous injection of the same solution used for the Dale experiment. By this method we were able to show in the same animal a positive uterine contraction and a typical anaphylactic death after intravenous injection. This was shown in 11 out of 39 animals.

This demonstrates that an animal sensitized by inhalation may develop a systemic as well as a local hypersensitiveness.

4175

Transmission of Fowl-Pox by Mosquitoes.

I. J. KLIGLER, R. S. MUCKENFUSS AND T. M. RIVERS.

From the Hospital of the Rockefeller Institute for Medical Research, New York.

The mode of transmission of certain virus diseases of chickens is still obscure. Fowl-pox as well as fowl plague are common diseases of the barnyard, both, according to reports, occurring during the spring and fall months. Many workers[1, 2] state that attempts to infect by contact or by ingestion of infected foodstuffs usually fail. These facts suggested the possibility of insect transmission.

Since fowl plague is not prevalent in this country our experiments were conducted with fowl-pox. Using *Culex* and *Aëdes* mosquitoes it was possible to transfer the disease from infected to healthy fowls with considerable regularity by interrupting the mosquitoes after they began feeding on infected areas of the diseased chicken and allowing them to complete their meal on the comb or wattles of healthy ones. Positive transmission of the disease was obtained even when the interval between feedings was 2 hours. As a rule,

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[1] Goodpasture, E. W., "Filterable Viruses," T. M. Rivers, Williams and Wilkins Company, 1928.

[2] Todd, C., I. *Brit. J. Exp. Path.*, 1928, ix, 19.

7 days after the feeding, typical minute vesicles appeared on the normal fowl wherever the proboscis of the insect had been inserted.

In one experiment transmission was obtained under conditions approximating those existing in nature. Two healthy and one infected chickens were placed in a box covered with fine-mesh copper screen. Four freshly hatched *Culex* mosquitoes were released in this box. After 7 days 2 typical discrete lesions appeared on the left wattle of one of the healthy fowls. In a control group without mosquitoes none of the chickens showed evidences of infection after a period of 17 days.

It seems, therefore, that where the lesions are superficial, as in fowl-pox, interrupted feedings by mosquitoes and possibly also by blood-sucking flies may play an important, if not the most important rôle in the dissemination of the disease. Our experiments thus far indicate that transmission of this kind is a mechanical transference of the virus; consequently it is not essential that the infectious agent be able to establish itself in the insect. In diseases where the virus is present in the circulation in high concentration, as in fowl plague, mechanical transference of the virus by interrupted feedings of insects may also be an important factor in the dissemination of such infections.

4176

Determination of Tertiary Dissociation Constant of Phosphoric Acid.

I. NEWTON KUGELMASS.

From the Department of Pediatric Research, The Fifth Avenue Hospital, New York.

Quantitative studies of the mechanism of calcification require a knowledge of the physico-chemical constants of the blood equilibria involved in the process. The tertiary dissociation constant of phosphoric acid, K_3, thus far available seems incompatible with the apparent dissociation constant pK_3 from the dissociation curve for the phosphates which is of an order of 1×10^{-12}.

The value of K_3 was determined experimentally from a study of aqueous systems of the tertiary sodium and potassium phosphates at equilibrium, from the standpoint of hydrolysis. The value thus obtained was correlated with calculations made from the data obtained by the electrometric titration of phosphoric acid.

The tertiary phosphates hydrolyze to a great extent, the secondary and primary salts inappreciably. In the mechanism of the first stage hydrolysis of the tertiary salts, the essential equilibrium, may be expressed ionically:

$$PO_4^{\equiv}+H_2O \rightleftarrows HPO_4^{=}+OH^-$$

Applying the law of mass action,

$$\frac{[HPO_4^-]\ [OH^-]}{[PO_4^{\equiv}]} = K_h \text{.........................} (1)$$

Where the brackets include the ion activities, and K_h is the activity hydrolysis constant. The degree of hydrolysis, h, is by definition,

$$h = \frac{[HPO_4^{=}]}{\gamma_B C} = \frac{[OH^-]}{\gamma_B C} \text{.........................} (2)$$

$$\text{and } 1-h = \frac{[PO_4^{\equiv}]}{\gamma_T C}$$

Where γ_B, γ_S, γ_T are the activity coefficients of hydroxide, secondary and tertiary phosphates of sodium or potassium respectively. Substituting the values of $[OH^-]$, $[HPO_4^{=}]$ and $[PO_4^{\equiv}]$ from these relations in Equation 1, we have

$$\frac{h^2 \cdot \gamma_B \cdot \gamma_S \cdot C}{(1-h)\ \gamma_t} = K_h$$

Also, the condition maintains that $[H^+] \cdot [OH^-] = K_w$. Dividing this by Equation 1,

$$\frac{[H^+]\ [PO_4^{\equiv}]}{[HPO_4^{=}]} = \frac{K_w}{K_h} = Ka$$

where Ka is the tertiary ion activity or true constant.

Purely prepared tertiary phosphates were made up to definite concentrations with conductivity water and kept in pyrex flasks. The hydrion concentrations of these solutions were measured potentiometrically in a thermostat maintained at 20° and 38° C. ± 0.02 respectively. The gas chain consisted of Pt—H_2—solution χ saturated KCl—HgCl—Hg. The hydrion concentration measurements were standardized daily against a 0.1 N HCl prepared from constant boiling acid. The pH is 1.085 at 20° and 1.09 at 38°, extrapolated from the activity coefficients determined by Noyes and Ellis. The calculated EMF values of the entire system against the acid standard are 0.3119 at 20° and 0.3022 volts at 38°. The average divergence was 0.003 pH. The observed voltages were corrected to one atmosphere of hydrogen.

The tertiary phosphates were recrystallized twice from distilled water free from CO_2 and again by adding an equal volume of 95% alcohol and cooling in ice water. Crystallization proceeds at the interface. The Na_3PO_4, 12 H_2O crystals are trigonal and the K_3PO_4, 2 H_2O are needle like. No attempt was made to prepare constant hydrates but the crystals were dried to remove free moisture and analyzed for water of hydration.

The value of tertiary dissociation constant of phosphoric acid determined from the hydrolysis of tertiary sodium and potassium phosphates respectively at 20° C. has been found to be 1.02×10^{-12}, and at 38° C. 1.48×10^{-12}.

The tertiary dissociation constant calculated from the data obtained by the electrometric titration of phosphoric acid and from formulas developed by Van Slyke, have been found to be 0.97×10^{12}, apparently at laboratory temperature.

4177

Effect of Immune Sera Upon the Phenomenon of Local Skin Reactivity to B. Typhosus Culture Filtrates.

GREGORY SHWARTZMAN.

From the Laboratories of the Mount Sinai Hospital, New York City.

In a previous communication[1] a phenomenon of local skin reactivity to *B. typhosus* culture filtrates was described. The reactivity was induced by skin injections of the filtrate. If 24 hours later an intravenous injection of the same filtrate was given to the rabbit there appeared an extremely severe hemorrhagic necrosis at the site of the previous skin injections. The mechanism of the phenomenon is not fully understood as yet, since no complete experimental comparison was made between this phenomenen and manifestations of bacterial allergy. There were found, however, certain features which, *considered together,* distinguish this phenomenon from the known phenomena of bacterial hypersusceptibility and the Arthur phenomenon. These features are: the local reactivity; the short incubation period necessary for local preparation of the skin; the short duration of the state of reactivity; the ability to induce the reactivity by a single injection and the necessity to make the second injection of the toxic agent by the intravenous route.

This report concerns the relation of the specific antisera to the skin preparatory factors of the observed phenomenon. There was a double purpose in these studies: first, to add new data leading to the elucidation of the mechanism of the phenomenon, and secondly, should there occur neutralization of *B. typhosus* skin preparatory factors by specific sera, to determine whether the phenomenon could be advantageously used for titration of the neutralizing properties of the sera. In order to fulfill this plan the effect of homologous normal and heterologous sera upon the skin preparatory factors of the phenomenon of local skin reactivity to *B. typhosus* culture filtrates was studied.

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[1] Shwartzman, G., *J. Exp. Med,* 1928, xlviii, 247.

Most of the sera were prepared by immunizing rabbits and goats with *B. typhosus* culture filtrates. The mixtures of filtrates with specific sera in various proportions were injected into the skin of the abdomen of the rabbits. Numerous experiments were performed with the following results:

In the majority of rabbits these mixtures failed to elicit any local skin reactivity. However, there were some rabbits in which the admixtures of homologous serum did not prevent the phenomenon of local skin reactivity. The neutralized reactions by far outnumbered the non-neutralized ones. On further analysis the reactions could be divided into 3 groups, namely: completely neutralized reactions (C.N.), partially neutralized reactions, (P.N.) and non-neutralized reactions (N.N.) Titrations of various batches of sera established the fact that the potency of a given serum bears a mathematical ratio to all the types of reactions. Thus one has to take into consideration all the data obtained, namely, the titer of serum producing complete neutralization in C.N. and P.N. rabbits as well as the number of rabbits belonging to each type of reaction. For each serum the total of rabbits was not less than 10.*

The studies on the normal sera brought out the fact that in majority of instances they failed to neutralize the *B. typhosus* skin preparatory factors of the observed phenomenon. These normal animals were able to respond with production of neutralizing substances if treated with the filtrates.

However, there were encountered some normal sera which gave certain evidence of neutralization of these factors in low dilutions. Such sera were found to contain agglutinins or both agglutinins and precipitins for *B. typhosus* in fairly high titer. There were no normal sera obtained as yet which neutralized the reactions and at the same time contained no agglutinins for *B. typhosus*. There were, however, sera which contained agglutinins or both agglutinins and precipitins which failed to give neutralization reactions.

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* The technique of such determinations with protocols of the results will be described elsewhere.

Several heterologous sera were also investigated. Non related sera were not able to neutralize the factors (Shiga, Scarlet fever, Erysipelas sera). Sera closely allied to *B. typhosus* sera (Para A and B) neutralized these factors in various proportions. The reactions also divided themselves into the same 3 types as described above (C.N., P.N. and N.N.). It is not known as yet whether the neutralization is a group reaction or the skin preparatory factors are identical with those of *B. typhosus*. It will be necessary to titrate a large number of various batches of sera in order to bring out the possible quantitative differences in the titers.

It appears from these studies that a new method is available for quantitative titration of substances in the serum which neutralize the skin preparatory factors of the phenomenon of local skin reactivity to *B. typhosus* culture filtrates. Emphasis should be laid on the fact that it is possible to control the individual susceptibility of rabbits to this phenomenon. This fact permits of considerable accuracy in quantitative titration of the sera. Attempts are under way to determine whether the proposed method can be applied to production of therapeutic sera.

Work is also in progress to determine the effect of specific sera upon the skin reacting factors introduced by the intravenous route.

4178

Stability of Luminous Substances of Luminous Animals.

E. NEWTON HARVEY.

From the Physiological Laboratory, Princeton University.

It is about ten years (1918) since I first collected the luminous ostracod crustacean, *Cypridina*, extracted the luminous materials, luciferin and luciferase, and set some of the extracts aside for later testing as to stability. The animals are collected and dried quickly over $CaCl_2$. When powdered and moistened a bright bluish luminescence appears which is as intense with the 1918 material tested in 1928 as it was in 1918. This powder contains both luciferin and luciferase and light results from mixing the 2 substances in water containing oxygen. Luciferin solution is obtained by making a hot water extract of *Cypridina*. Heating destroys the luciferase, which can be extracted from *Cypridina* with cold water. The luciferin, which dissolves at the same time, is allowed to oxidize in the air, leaving luciferase alone in solution.

Sterile luciferin extracts made in 1918 and kept under a 3 inch layer of vaselin, to prevent oxidation, gave a good luminescence when luciferase was added to them in 1928. So long as oxidation is prevented, luciferin in water solution appears perfectly stable. A flask of luciferin solution was evacuated and the glass sealed. This flask was then heated in a water bath at 100° C. for one hour and set aside, sealed, January 22, 1924. On October 16, 1928 the flask was opened to the air. No luminescence appeared on opening, but when luciferase was added, a brilliant light appeared. Evidently boiling does not destroy luciferin if air is absent. Absolute alcohol extracts of luciferin kept in air show no luminescence after a few weeks when mixed in small quantity with luciferase solution. A slow oxidation of luciferin must take place.

Luciferase solution cannot be heat sterilized and if allowed to stand unsterilized, undergoes putrefaction and the luciferase disappears after a few weeks. A solution preserved with chloroform retains the power to luminesce when mixed with luciferin for several months but the luciferase gradually disappears and a sample 10 years old gave no luminescence with luciferin. Preserved with saturated NaCl, luciferase also gradually deteriorates, but a sample over 10 years old gave a faint luminescence with luciferin. On the other hand luciferase precipitated from solution with alcohol, the precipitate dried and kept over $CaCl_2$ for 10 years, gave a good luminescence when mixed with luciferin solution.

I conclude that dry luciferin and luciferase can be kept unharmed for long periods and that an aqueous luciferin solution is also stable in absence of oxygen, but that luciferase undergoes destructive changes in water solution.

4179

On Hemagglutination by Tumor Extracts.

K. LANDSTEINER.

From the Laboratories of The Rockefeller Institute for Medical Research, New York.

A report was made years ago on a distinct agglutinating action of extracts of tumors, of human and animal origin, on rabbit erythrocytes.[1] The question was raised whether the active substance might possibly be related to growth stimulating principles.

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[1] Landsteiner, K., *Wien. klin. Wochenschr.*, 1908, S. 1549.

The agglutinating activity of the extracts disappears rather rapidly on standing and this property in itself differentiates the active substance from serum agglutinins, an inference probable also for other reasons. The agglutination phenomenon is shown also by Rous tumors.

Recently Mueller[2] described experiments which showed that the active principle of the Rous sarcoma deteriorates quickly on account of oxidation and that it can be preserved by the addition of cysteine and protection from the air. These results suggested similar experiments with the agglutinating extract of tumors. It was found that the deterioration of the agglutinating activity of tumor extracts (mouse sarcoma and Rous tumor) is also very markedly delayed when cysteine (1:500) is added to the solution and still more when the latter is covered with a layer of liquid paraffin. Thus the action is obviously different from agglutination by serum. A distinct agglutinating action, less intense than that of the tumors, was found in extracts of mouse embryos and mouse placenta.

Saline extracts of normal organs, as stated previously, did not show the agglutination phenomenon. Experiments are being made, using cysteine, in order to investigate whether a similar, though weaker, agglutinating action can be detected also in extracts of normal organs.

4180

Penetration of Alkaloids Into Vacuoles of Living Cells.

MARIAN IRWIN.

From the Laboratories of The Rockefeller Institute.

The rate of penetration of brucine (from a solution of brucine sulphate) into the vacuole of a living cell of *Nitella* is much greater with the external solution at pH 9.3 than at pH 5.5. At pH 9.3 brucine accumulates in the sap and becomes more concentrated than in the external solution. As brucine penetrates, the pH value of the sap in the vacuole in increased. (The brucine is tested with nitric acid and the pH value with indicators.)

When cells exposed to brucine sulphate solution are transferred to a buffer solution containing no brucine the rate of exit of brucine from the vacuole is greater when the buffer solution is at pH 5.5

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[2] Mueller, J. H., *J. Exp. Med.*, 1928, xlviii, 343.

than at pH 9.3. The difference in the rate caused by the variation in the external pH value is less marked with exit than with penetration.

As brucine comes out of the vacuole the pH value of the sap is decreased.

The same result is obtained with penetration of brucine into the "vacuole" of an artificial system consisting of a layer of chloroform placed between the brucine sulphate solution and the sap in a U-tube.

The results indicate that brucine goes in and out of the vacuole of a living cell primarily in form of free base, but the mechanism of penetration is somewhat different from that of the exit, just as was shown previously with cresyl blue.[1] The accumulation of brucine is chiefly caused by the transformation of free base into the salt by the sap, but in some cases is due partly to the formation of a very slightly soluble compound of brucine with some constituent of the sap. The results support the multiple partition coefficient theory[2] which states that the cell controls the rate of penetration and exit of a substance as it would if it were made up of a non-aqueous layer lying between the external solution and the vacuolar sap, the rate of diffusion of a substance being partly dependent on its partition coefficient at each phase boundary.

The same results were obtained with codeine hydrochloride.

4181

Adrenalin and Fatiguability of Muscle of Adrenalectomized Rats.

L. B. NICE, D. S. GREENBERG AND S. L. GREENBERG.

From the Physiological Laboratories, Ohio State University.

A series of adrenalectomized rats was given subcutaneously 1 cc. of 1:50,000 adrenalin chloride (Parke Davis & Company) daily for periods of 7 days to 14 weeks. The animals were then urethanized and a fatigue curve of a gastrocnemius muscle obtained by a modification of the Gans and Miley method.[1]

The results are shown in Table I.

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[1] Irwin, M., *J. Gen. Physiol.*, 1926-27, x, 75.

[2] Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1927, xxv, 127.

[1] Gans, H. M., and Miley, H. H., *Am. J. Physiol.*, 1927, lxxxii, 1.

TABLE I.

Absolute muscle force and total work done by gastrocnemius muscle.

Animal number	Absolute muscle force per gm. of muscle	Absolute muscle force	Total work	Wgt. of muscle	Work per gm. of muscle	Age of rat	Wgt. of rat	Time given adrenalin
	gm.	gm.	gm. cm.	gm.	gm. cm.	days	gm.	days
303 ♀	753.3	678	12,800	0.9	1,422	277	190	84
307 ♀	226.3	430	330,387	1.9	173,888	271	190	97
84 ♀	836.3	920	288,618	1.1	262,380	218	120	11
338 ♂	964.3	1350	62,244	1.4	44,460	256	172	7
82 ♀	740.0	1110	112,320	1.5	74,880	255	181	7
91 ♂	607.7	790	10,080	1.3	7,754	266	220	7
A ♂	758.3	910	4,077	1.2	3,398	75	98	8
B ♂	780.8	937	32,273	1.2	26,894	75	150	9
E ♂	1160	1160	3,182	1.0	3,182	75	150	9
D ♂	400	400	151	1.0	151	75	100	9
C ♂	761	784	70,606	1.0	69,222	75	100	9
Average for 11 animals	726.2	860.8	84,249	1.2	60,694		152.7	

The amount of work per gm. of muscle done in our series varied between 151 and 262,380 gm. cm., while with the adrenalectomized rats of Gans and Miley it varied between 1200 and 206,400 gm. cm. The average work per gm. of muscle of our rats amounted to 60,694 gm. cm., which is slightly less than that of their adrenalectomized rats—64,200 and only one-sixteenth of the average of their controls—1,015,300 gm. cm.

There is no evidence of increased work done by the muscles of our rats after the daily administration of adrenalin.

4182

Guanidine Retention and Calcium Reserve as Antagonistic Factors in Carbon Tetrachloride and Chloroform Poisoning.*

A. S. MINOT AND J. T. CUTLER. (Introduced by Paul D. Lamson.)

From the Department of Pharmacology, Vanderbilt University, School of Medicine.

An earlier note[1] reported the rather empirical observation that dogs on a lean meat diet can be made either highly resistant or susceptible to carbon tetrachloride intoxication by adding or withholding calcium salts for a few days previous to the administration of carbon tetrachloride. Furthermore calcium therapy was found highly effective in the treatment of cases of poisoning.

The intoxication produced in susceptible dogs was described in the earlier note and is characterized by gastro-intestinal irritation, nervous disturbances and convulsions, followed by weakness, depression, and death. There is a retention of bile pigments in the blood and a hypoglycemia which usually becomes extreme before death and which seems in most cases to be the immediate cause of death. The level of total calcium in the blood is usually within normal limits, nevertheless either a lack of or excessive demand for calcium ions seems to be indicated by the relief afforded by the furnishing of extra available calcium. It was at first suggested that a depression of ionized calcium due to combination with bile pigments might account for the apparent calcium lack. This is probably one factor in the need for extra calcium, but further work has indicated that it is probably not the only or even the most important factor.

A similar toxic picture can be produced by the administration of guanidine compounds. Following subcutaneous doses of 250 mgm. of guanidine hydrochloride per kilo body weight, dogs show fibrillary twitching of muscles, tetanic convulsions, gastro-intestinal irritation with bloody vomitus and diarrhea, with usually a period of extreme depression and weakness for some time before death, which occurs 12 to 15 hours after the drug is injected. Four or 5 hours after the guanidine is administered there is a marked fall in blood sugar and the hypoglycemia becomes extreme unless a diet very rich in calcium and carbohydrate has been fed for some time preceding the experiment. In this intoxication as well as in carbon tetrachloride poisoning, relief is afforded by the prompt intravenous administration of calcium salts.

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* This investigation is one of a series of studies being made under the direction of Dr. P. D. Lamson on the pharmacology and toxicology of carbon tetrachloride. The work is being carried on with the support of the International Health Board.

[1] Minot, A. S., PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 617.

Further evidence of a more fundamental similarity in the 2 conditions was furnished by the determination of guanidine in the blood of large numbers of animals. A carefully controlled adaptation of the method for guanidine determination described by Major and Weber[2, 3] was used. Guanidine and sugar determinations were made on samples of blood taken before and at intervals after the administration of the toxic drug in the following types of intoxication: I. Following the oral administration of 4 cc. CCl_4 per kilo to dogs on the following diets: (a) on a diet of meat without extra calcium; (b) on a diet of meat with extra calcium; (c) on a diet of bread and milk. II. Following the oral administration of 4 cc. CCl_4 per kilo + 4 cc. alcohol per kilo to dogs on the diets described above. III. Following the inhalation for 1½ hours of chloroform vapor of a concentration sufficient to maintain complete anesthesia. IV. Following the subcutaneous administration of varying doses of guanidine hydrochloride.

From these studies it was found that both carbon tetrachloride and chloroform cause some interference with normal metabolism which results in a tendency for guanidine to accumulate in the blood. Until more is known of the origin and normal disposal of guanidine nothing definite can be concluded regarding the site or nature of this interference. From the similar effects produced by 2 well known liver poisons one is tempted to attribute the disturbance to a failure on the part of the damaged liver to contribute some necessary step in normal metabolism. This point has, however, not yet been proved. It seems probable that precursors of guanidine are abundant in meat since the increase in guanidine following the administration of carbon tetrachloride is largely avoided if the animals are fed a bread and milk diet. There is also, however, an endogenous source of guanidine because in the more severe intoxications produced by giving alcohol with carbon tetrachloride and by chloroform there is an accumulation of guanidine even when meat is not eaten.

Within a few hours after an appreciable increase of guanidine in the blood the typical symptoms described above appear and there is a fall in blood sugar. The hypoglycemia is more extreme and usually proves fatal in the animals on a low calcium diet. The difference in the severity of intoxication in the high and low calcium groups

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[2] Major, R. H., and Weber, C. J., *Johns Hop. Hosp. Bull.*, 1927, xl, 87.

[3] Major, R. H., and Weber, C. J., *Arch. Int. Med.*, 1927, xl, 891.

on the meat diet seems to lie in the difference in the severity of the subsequent hypoglycemia rather than in the concentrations of guanidine in the blood. Further evidence that the additional dietary calcium is responsible for the avoidance of dangerously low blood sugar levels was furnished by other experiments in which rapidly falling sugar levels were restored and maintained at a safe level by the administration of calcium salts. This effect on blood sugar is, however, not the only basis for the relief afforded by calcium because when a normal blood sugar level is maintained by the administration of dextrose the nervous symptoms persist unless calcium is also given.

The more severe intoxications produced by carbon tetrachloride given with alcohol, or by chloroform, apparently do not differ qualitatively from that produced by carbon tetrachloride alone. There is a higher and more rapid rise in guanidine in the blood which occurs to some extent even when meat is not eaten. This rise is soon followed by severe hypoglycemia and death. Calcium has the same antagonistic action here as in cases of carbon tetrachloride poisoning, though in these cases the need is more imperative. Mere preliminary feeding of a high calcium diet is usually inadequate to prevent serious symptoms of intoxication. When calcium feeding is supplemented by persistent calcium and dextrose therapy marked temporary relief is afforded and permanent cures can often be effected unless gastro-intestinal hemorrhages become uncontrollable.

Of most importance in establishing a causal relationship between the symptoms seen in any of the cases just described and the observed concentrations of guanidine in the blood are the results obtained from the group of animals receiving guanidine hydrochloride. When similar symptoms were produced quite comparable concentrations of guanidine were found in the blood whether the drug administered to produce the intoxication was carbon tetrachloride, chloroform, or guanidine hydrochloride.

The reason for the protective and curative action of calcium salts reported in our earlier paper[1] is apparently due to the antagonism between calcium and the guanidine which tends to accumulate in the blood. Practical suggestions for the safe use of carbon tetrachloride would, therefore, emphasize both the advisability of avoiding meat, and of furnishing adequate dietary carbohydrate and calcium for several days before and after the administration of the drug. Should cases of intoxication occur calcium and dextrose therapy would be indicated and in our work with dogs has proved very effective.

4183

Influence of Arsenic, Ferrous Sulphate and Copper Sulphate on Rats Furnished A Vitamin A-Free Diet with Iron Added.

F. E. CHIDESTER* AND A. G. EATON.

From the Department of Zoology, West Virginia University.

In order to ascertain the extent to which minerals may act as catalyzers and replace the so-called "vitamins", we have been performing experiments with a number of chemicals added in minute doses to the food of experimental rats, showing unmistakable signs of avitaminosis. Our experiments with iron and iodine[1] having proved successful, it was deemed wise to try preliminary experiments in which copper was exhibited in the presence of iron and arsenic. In a series of experiments with 15 negative control and 8 positive control rats, 6 other animals were placed on a diet that included ferrous sulphate, iron iodide, arsenic and copper sulphate added to the Sherman A-free diet No. 380.

FIRST LOT: 2 male animals received daily 1 drop of a solution of copper sulphate, such that it added to their food 0.01 mgm. of copper. They received 1 drop of ferrous sulphate solution containing 0.5 mgm. of iron and 0.01 mgm. of ergosterol, which had been irradiated together with a small quantity of the Sherman diet No. 380. They also received in the drinking water, 1 cc. of dilute Fowler's solution of arsenic containing 0.379 mgm. of arsenic.

Beginning with an average weight of 42 gm., they reached a maximum weight of 175 gm. at the end of 9 weeks. After fluctuating around 170 gm. for 2 weeks a rapid decline resulted in death at the end of 13 weeks. Autopsies showed that in one case the left ear and left adrenal were infected while the other specimen had infected kidneys, testis and gall bladder.

SECOND LOT: In another lot of 4 females, receiving Sherman diet No. 380 and irradiated ergosterol, 1 drop of ferrous sulphate solution and 5 drops of dilute syrup of the iodide of iron, the experiments were also unsatisfactory. Averaging 53 gm. at the beginning of the experiment, they reached a maximum of 160 gm. at the end of 12 weeks. For 7 weeks they fluctuated around 157 gm. The results indicated storage but no benefit. Autopsies showed 2 with

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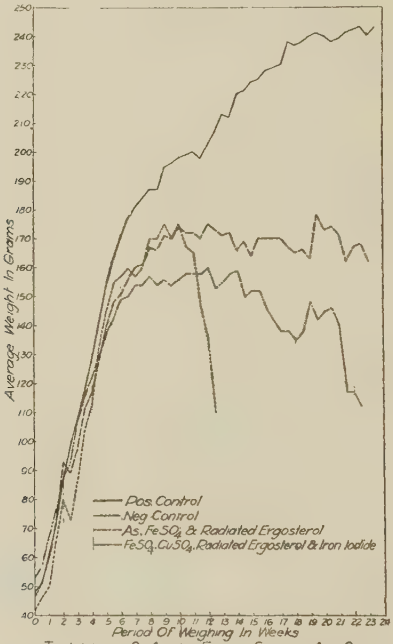
Subsidized by the Purnell Fund and the Merrill-Soule Company.

[1] Chidester, F. E., Eaton, A. G., and Thompson, G. P., *Science*, 1928, lviii, 432.

infected gall bladders, 3 with infected lungs, 1 with infected thyroid and 1 with no apparent infections.

Hart and associates[2] found that .01 mg. of copper sulphate given daily to rats in addition to 0.5 mg. of iron was effective in curing anemia in from 2 to 3 weeks. Our results differ in that the effect noted by the Wisconsin investigator did not persist. (See graph.)



THE INFLUENCE OF ARSENIC, FERROUS SULPHATE AND COPPER SULPHATE ON RATS FURNISHED A VITAMIN A FREE DIET

Our animals were run on vitamin A deficient diet for over 3 months and naturally, even if the minerals furnished did act as catalyzers and make available the stored food, the experiments cannot be compared to one in which an *adequate* diet was furnished.

It is interesting to note, however, that in anemia produced by bleeding, Whipple[3] and associates at Rochester University found that iron was more potent than either copper or zinc.

We hope later to find the optimum minute amount of copper that will be effective in combination with other minerals that we have recently established as catalyzers of value in avitaminosis.

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[2] Hart, E. B., Steenbock, H., Waddell, J., and Elvhjem, *J. Biol. Chem.*, 1928, lxxvii, 797.

[3] Whipple, G. H., Robscheit-Robbins, F. S., Elden, C. A., and Sperry, W. M., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 748.

4184

Influence of Modified Fowler's Solution of Arsenic on Rats Receiving Vitamin A-Free Diet with Iron Iodide Added.

F. E. CHIDESTER,* A. G. EATON AND C. P. THOMPSON.

From the Department of Zoology, West Virginia University.

About a year ago we conceived the idea that certain minerals, particularly iodine and iron, might be used as substitutes for Vitamin A. Rather extensive experiments with these minerals have been performed and the results with 57 rats are to appear soon in *Science.*

Having established the optimum dosage of iron iodide for rats on the Sherman A-free diet, it was deemed advisable to test the action of potassium arsenite, administered in the form of a Fowler's solution of arsenic. The formula used did not include the oil of lavender ordinarily incorporated as it was feared that contamination with vitamin A might result.

The animals used were agouti and pink-eyed yellow rats, both extremely resistant stocks, described elsewhere. As in other experiments performed in our laboratory, the young rats, approximately 30 days of age, were placed in individual round cages of the Sherman type and furnished a plentiful supply of distilled water. Individual records were kept of the foods consumed and weights were recorded twice each week. Autopsies were performed as soon as possible after death.

Twenty-eight animals were used in the experiment. The control animals used in the experiment were 23 in number, 15 negative and 8 positive. The positive controls received 1 drop of cod liver oil daily, whereas the negative controls were fed only Sherman A-free diet No. 380. Five animals placed on the standard Sherman A-free diet No. 380, received in addition, 3 drops daily of a solution of iron iodide which was added to their food. Each animal received 0.01 mgm. of irradiated ergosterol which was fed daily mixed with 1 gm. of the deficiency diet No. 380. Fowler's solution of arsenic was administered in the drinking water in such strength that each cubic centimeter contained 0.379 mgm. of arsenic.

At no time in the experiment did the average weights of the experimental animals reach that of the negative controls. Starting with an average weight of 40 gm., they reached a maximum of 169

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* Approved for publication by the Director of the West Virginia Experiment Station, as Scientific Paper No. 69.

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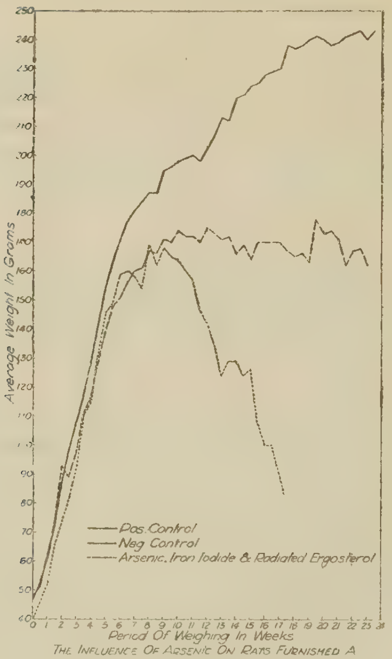

THE INFLUENCE OF ARSENIC ON RATS FURNISHED A VITAMIN A FREE DIET WITH IRON IODIDE ADDED

gm. at the end of 8½ weeks. After fluctuating around 160 gm. for 2 weeks the weights declined rapidly until death, which ensued at the end of 18 weeks. Declination in weight was much more rapid than in the negative controls.

Autopsies showed that 4 of the animals had either liver or gall bladder infection, 2 had badly infected thyroids, one had an infection in the right ear, while one had a remarkably hypertrophied right adrenal.

The literature is already burdened with presumably authoritative papers that include separate graphs of each of the 3 animals in a series. And the experiments have been stopped at the end of 3 or 4 weeks. Physicians are familiar with the temporary improvement induced by arsenic in anemia. While we realize that our results were obtained with too few animals, we should like to point out that the experiment ran for 18 weeks.

Digestive disturbances are common when arsenic is given to people in the presence of iron. It is therefore not surprising that our preliminary dosage was unsatisfactory in the case of experimental animals weakened by deficiency in "Vitamin A". Our success in furnishing iron and iodine as catalytic agents of importance in avitaminosis may induce us to try further experiments with smaller doses of arsenic as iron cacodylate.

4185

Dissociation of a Streptococcus Hemolyticus.

F. B. JENNINGS, JR. (Introduced by S. A. Petroff.)

From the Research and Clinical Laboratory, Trudeau Sanatorium, Trudeau, N. Y.

A hemolytic streptococcus was isolated from 1 of 50 guinea pigs dying during an epidemic of streptococcus infection. The organism under consideration is also pathogenic for white mice, white rats and rabbits. The plates made from the heart's blood of the original guinea pigs showed for the most part colonies of a typical virulent streptococcus, except for 2 colonies which were flat, translucent, yellowish and conspicuously large. After fishing and replating these 2 colonies, the above characteristics were accentuated except for the fact that they were less flat. In broth the growth was homogeneous in contradistinction to the flocculent growth of the ordinary virulent type of hemolytic streptococcus.

The organisms from the particular colony under discussion have certain unusual characteristics. Apparently they remain true to type, no matter how they are cultivated or passed through animals. They also maintain their virulence after standing at room temperature in plain broth for 2 or 3 months and on repeated subcultivation. The virulence is such that ¼ cc. of a 16 hour growth diluted 1-100,000,000 will kill mice.

The organisms of the conventional colony mentioned above behave, as far as virulence is concerned, as any hemolytic streptococ-

TABLE I. *Representative Virulence Tests.*

	Dilution of Cultures.					
	1-100	1-10,000	1-100,000	1-1,000,000	1-10,000,000	1-100,000,000
Conventional type of colony after daily subculturing in broth for 2 weeks.	S	S		S		
Conventional type of colony after direct intraperitoneal passage through 8 mice.		2		2	2	S
Flat type of colony after daily subculturing in broth for 2 weeks.		2		2	2	3
Flat type of colony after direct intraperitoneal passage through 5 mice.				1	2	2

The mice received ¼ cc. of broth diluted 16 hour culture intraperitoneally.
"S" indicates a 96 hour survival.
The numbers indicate the day of death after inoculation.

FIG. 1.

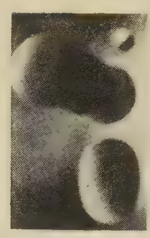

FIG. 2.

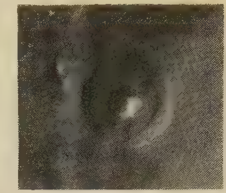

FIG. 3.

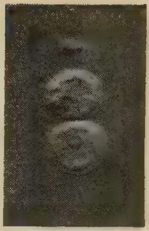

FIG. 4.

Fig. 1. Typical large flat colony compared to the ordinary virulent streptococcus colony. *Fig. 2.* Variant forms of large flat colony and a virulent streptococcus colony. *Fig. 3.* Flat colony with central nipple and irregular raised periphery as may be seen after 48 hours. *Fig. 4.* Flat colony showing errosion which may also occur after 48 hours.

cus. However, upon cultivation in broth of a pH 6.5 and upon rapid mouse passage a few of the flat, translucent colonies eventually appear. On mouse passage they are recovered first from the peritoneal exudate rather than the heart's blood and apparently always predominate in the former. The organisms forming the flat colonies were found to be encapsulated when taken directly from the mouse, in contrast to non-encapsulated forms of the ordinary virulent streptococcus under similar circumstances.

There is some variation in the appearance as well as the behavior of these large colonies on different sorts of solid media. Agar enriched with some body fluid seems to stabilize them. Often on ordinary agar the colonies are seen to develop areas of erosion. Opacities also appear, as well as a central nipple and an irregular raised periphery.

4186

The Hyperglycemic Effect of Vasopressin, Oxytocin and Pituitrin.

E. M. K. GEILING AND C. A. EDDY. (Introduced by J. J. Abel.)

From the Pharmacological Laboratory, Johns Hopkins University.

Doctor Oliver Kamm and his associates kindly furnished us with experimental lots of their vasopressin and oxytocin and we were enabled to study in normal unanesthetized dogs and rabbits the changes in blood sugar following the intravenous injection of these 2 principles, as also the commercial pituitrin (obstetrical, Parke, Davis & Co.). We found that vasopressin, oxytocin and pituitrin caused a very definite hyperglycemia. The rise of blood sugar with oxytocin is more prolonged but not as high as with vasopressin. The hyperglycemia after pituitrin is intermediate in degree between that of vasopressin and oxytocin. These 3 substances behave differently in their hyperglycemic activity after treatment with 2 N sodium hydroxide in the cold. In the case of vasopressin the sugar raising property is practically completely destroyed whereas the oxytocin and pituitrin are only partially injured in respect to this property. The extent of destruction appears to be less with pituitrin than with oxytocin. This may be due to the larger amount of protein present in the pituitrin.

4187

Orientation of Cylindroid Particles in Gelatin-Serum Gels.

HAROLD A. ABRAMSON. (Introduced by R. Chambers.)

From the Kaiser Wilhelm Institute for Physical Chemistry and Electrochemistry, Berlin-Dahlem.

The phenomenon here described has been observed during the study of the cataphoresis of red cells and rouleaux in gelatin-serum sols and gels. The method of preparation of the suspension has been described elsewhere.[1]

The red cells of the horse, since they do not become coated with proteins in the manner of quartz particles when suspended in a soft gelatin-serum (2 to 3% gelatin) gel, have the remarkable property of migrating through a protein sol or gel. If cylindroids, consisting of red cells in rouleaux formation, are moved back and forth through such a sol or gel, by virtue of their cataphoretic mobility, no orientation of these cylindroid bodies occurs during the cataphoretic migration.

At the same time the gelatin and serum protein micellae are also in movement, as well as the liquid in the cataphoresis cell.[2] As gelation proceeds, the speed of the cylindroid bodies becomes much slower. In spite of the fact that the cylindroids have been moved back and forth in the cell over a considerable period, *i. e.*, 10 minutes, and even though they are apparently subjected to a laminary liquid stream, the orientation of the cylindroids is still at random with a tendency, however, to be placed in planes parallel to the horizontal plane of the cataphoresis cell. The current is now discontinued and gelation is permitted to proceed. After a considerable time interval (about 24 hours) during which time a stiff gel is formed, it is found that the cylindroid particles have been oriented so that their lengthwise axes are parallel to the lines of the current flow.* It is to be emphasized that the orientation takes place after the electric current has ceased to flow.

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[1] Freundlich and Abramson, *Z. Phys. Chem.*, 1928, cxxxiii, 52. Abramson, *J. Gen. Phys.*, 1928, xi, 743.

[2] Abramson, "Colloid Symposium Monograph No. 6," in press.

By making a more concentrated gel, one may observe the same phenomenon take place more rapidly when the sol and gel are sucked back and forth in the cell. For some time during gelation the orientation is at random when, suddenly, the cylindroids become oriented in the direction of the flow.

Hatschek has observed, in gelatin gels, the orientation of macroscopic lens-shaped gas bubbles perpendicular to the axis of an applied pressure. The same has been here observed for microscopic rouleaux arranged in parallel before the pressure is applied. Under such conditions, sucking on the cataphoresis cell may produce with proper gel consistency an orientation of the rouleaux perpendicular to their previous direction.

These experiments support the view that gelatin micellae form aggregates in thread-like arrangement in gel formation. The electrical and mechanical forces most probably produce an arrangement of these threads similar, say, to that which is known to take place in vanadium pentoxide sols under similar conditions. A gelatin gel which orients in parallel cylindroid particles previously arranged at random should be double refracting and may perhaps give an X-ray pattern. With increasing age of gel, the elasticity of fibers or threads making up the gel structure must increase and their length and diameter should become greater. During gelation, orientation is in all likelihood produced by the increase of directed intra-gel tensions, the direction of which is manifested by the rotation of the irregularly arranged cylindroid particles.

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* This has been observed in 3 out of 6 fairly similar although not identical experiments.

4188

Anterior Lobe Substance, the Thyroid Stimulator. I. Induces Precocious Metamorphosis.

E. UHLENHUTH AND S. SCHWARTZBACH.

*From the University of Maryland Medical School.**

In previous work[1] we found that Armour's commercial anterior lobe powder contains a specific substance which, when injected intraperitoneally, not only induces precocious metamorphosis of the injected salamander larvae *(Ambystoma tigrinum)*, but alone among other substances tested (inorganic iodine, thyroid substance, adrenalin, pilocarpin) causes a typical display of the complete functional phase of the thyroid gland of the injected larvae, such as takes place normally during metamorphosis. We concluded that anterior lobe is the extrinsic thyroid stimulator for which one of us has been searching since 1919, and that it causes metamorphosis of the amphibian larvae only indirectly, by forcing the thyroid to excrete. The existence and action of an extrinsic thyroid stimulator (excretor substance) has been shown in a long series of experiments,[2] but the stimulator itself could not be found until 1926.[1] Hogben[3] and later Spaul[4] had found that intraperitoneal injection of an extract made from Armour's anterior lobe powder and other commercial anterior lobe products induces precocious metamorphosis of the Mexican axolotl. But Smith claimed[5, 6] that the metamorphosis-accelerating action of Armour's commercial anterior lobe product is the effect, not of anterior lobe substance, but of a very high iodine content of the commercial product and probably of an admixture of thyroid substance. Spaul[7] demonstrated that an extract prepared from fresh anterior lobe had the same effect as Armour's anterior lobe powder, provided that the anterior lobe is separated from the posterior lobe immediately after the death of the bovine donator. Contamination of the anterior lobe by the posterior lobe takes place otherwise and such extracts are not only ineffective, but may inhibit metamorphosis. Smith,[6] however, insists that the only active extract to be obtained from anterior lobe must be prepared from the fresh gland according to the method described by Evans and Long.[8] Intraperitoneal injection of such extract *retards* metamorphosis.

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* This work was made possible by the "Julius Friedenwald Fund for Research" of the University of Maryland Medical School.

[1] Uhlenhuth, E., and Schwartzbach, S., *Anat. Rec.*, 1926, xxxiv, 119. *Brit. J. Exp. Biol.*, 1927, v, 1.

[2] Uhlenhuth, E., *J. Gen. Physiol.*, 1919, i, 473.

[3] Hogben, L. T., *Proc. Roy. Soc.*, B., 1923, xciv, 204.

[4] Spaul, E. A., *Brit. J. Exp. Biol.*, 1924, ii, 33.

[5] Smith, P. E., and Smith, I. P., PROC. SOC. EXP. BIOL. AND MED., 1922, xx, 51.

[6] Smith, P. E., *Brit. J. Exp. Biol.*, 1926, iii, 239.

[7] Spaul, E. A., *Brit. J. Exp. Biol.*, 1925, ii, 427.

In this article will be described the effects upon metamorphosis of extracts prepared in our own laboratory. Aided by 3 assistants, Messrs. M. Dawson, P. C. Jett and H. S. Shelley, we collected the bovine glands in one of the Baltimore abattoirs.† Separation of the 2 lobes was effected one hour after death (by bleeding) of the donators (cows and oxen), the glands cut into small pieces, dried, at room temperature, on glass plates and put away into bottles. Before use the glands were subjected to thorough grinding and desiccation; the fat was not extracted. Extracts were prepared by the method of Spaul, neutralized and injected intraperitoneally 3 times a week.

Larvae of the Utah axolotl (raised from eggs in the laboratory and kept under identical conditions) were injected beginning at an age of 91 days. The average body length was 33.7 mm. (controls) and 35.1 mm. (experimentals). The body length of the metamorphosing control larvae exceeded that of the metamorphosing experimentals by 20 mm.; the experimentals metamorphosed 36 days before the controls. In another experiment, with the larvae of the Long Island *Ambystoma tigrinum,*‡ raised from the eggs in the laboratory and kept under identical conditions, the body length of the metamorphosing experimentals was 10 mm. less than that of the metamorphosing controls. In a third experiment, with Long Island larvae of *A. tigrinum,* the body length of the metamorphosing controls exceeded that of the metamorphosing experimentals by 9 mm. In a fourth experiment with the larvae of the Utah axolotl, in which only one control and 2 experimentals were used the body length of the experimentals was 10.6 mm. less than that of the controls, when metamorphosis took place.

Intraperitoneal injections of Ringer solution and muscle extract had no effect on metamorphosis.

These experiments prove conclusively that anterior lobe substance, even when dried anterior lobe powder is used, enforces promptly the metamorphosis of Urodelan larvae. Evidently Smith, who in his earliest experiments on the larvae of tailless amphibians, obtained results comparable to those reported here, is working now, in his experiments on mammals and urodelan amphibians, with anterior lobe products which are polluted by posterior lobe substance. The latter circumstance seems to be of considerable importance as some of the effects in mammals, claimed by Smith to be caused by anterior lobe substance, may be really caused by admixture of posterior lobe.

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[8] Evans, H. M., and Long, J. A., *Anat. Rec.*, 1921, xxi, 62.

† We wish to express our gratitude to the Kaufman Packing Co. of Baltimore, for the most generous assistance which they have given us throughout our work.

‡ The spawn of the Long Island larvae was secured through the kindness of Dr. S. C. Bishop, Department of Biology, University of Rochester, N. Y., who collected it for us.

4189

Anterior Lobe Substance, the Thyroid Stimulator. II. Effect of Feeding Anterior Lobe Upon Amphibian Metamorphosis.

S. SCHWARTZBACH AND E. UHLENHUTH.

*From the University of Maryland Medical School.**

In 1921 one of us showed that feeding fresh anterior lobe of the hypophysis of cattle has no effect on the metamorphosis of *Urodelan* amphibian larvae.[1] As Smith[2] obtained precocious metamorphosis by feeding Armour's anterior lobe powder, he felt certain that this preparation is not pure anterior lobe powder, but owes its action to the presence of iodine or thyroid substance in it.

We fed anterior lobe powder to larvae of the Utah axolotl, raised from eggs in the laboratory and kept under identical conditions. A 1928 sample of Armour's commercial anterior lobe powder and anterior lobe powder prepared in our own laboratory by the method described in Article I of this series were used.

In one experiment 2 controls metamorphosed at an average body length of 58.2 mm., average age 141 days; 3 experimentals fed on our own anterior lobe preparation metamorphosed at an average body length of 63.7 mm., average age 140 days; and 2 experimentals fed on Armour's anterior lobe powder metamorphosed at an average body length of 65 mm., average age 135 days; one of the latter 2 animals attained the largest size before metamorphosis (68.1 mm.) among the entire 1928 material. The feeding lasted for a period of approximately 102 days. Feeding of a 1928 sample of Armour's

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* These experiments were carried out with the aid of the "Julius Friedenwald Research Fund" of the University of Maryland Medical School.

[1] Uhlenhuth, PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 11.

[2] Smith, P. E., *Brit. J. Exp. Biol.*, 1926, iii, 239.

thyroid powder, in the same kind of larvae, caused metamorphosis 14 days (at the latest) after the beginning of the experiment.

Neither our own nor Armour's preparation of anterior lobe had any effect on the metamorphosis of the salamander larvae when administered by mouth, while they proved most potent when injected intraperitoneally. Armour's preparation behaved in this respect entirely as our own preparation, and both differed from Armour's thyroid powders.

4190

Anterior Lobe Substance, the Thyroid Stimulator. III. Effect of Anterior Lobe Substance on Thyroid Gland.

E. UHLENHUTH and S. SCHWARTZBACH.
*From the University of Maryland Medical School.**

In previous work[1] we found that intraperitoneal injection of an extract made from Armour's anterior lobe powder causes the thyroid gland of the larvae of *Ambystoma tigrinum* to display its functional phase in a typical manner long before it is displayed in normal animals and linked with the progress of metamorphosis in the same way as it is in normal metamorphosis. Exactly the same result we obtained by injecting, intraperitoneally, an extract of anterior lobe powder prepared in our own laboratory by the method described in Article I of this series. Illustrations are required to demonstrate satisfactorily these results and will be published in a more complete article. The main results will be summarized here.

The animals used are the same as those discussed in Article I of this series. Larvae of the eastern as well as of the western race (Utah axolotle) of *Ambystoma tigrinum* were examined. The thyroids of 16 experimentals and 11 controls were examined. The thyroids were studied fresh, stained or unstained, by a method described fully in a previous article.[2] Three representative thyroids will be discussed here.

In one larva (CCXCIV b 2) the cells of the thyroid were found distinctly changed towards increased functional activity, although

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* These experiments were made with the support of the Julius Friedenwald Fund for Research of the University of Maryland and of the Elizabeth Thompson Science Fund of Harvard University.

[1] Uhlenhuth, E., and Schwartzbach, S., *Anat. Rec.*, 1926, xxxiv, 119. *Brit. J. Exp. Biol.*, 1927, v, 1.

[2] Uhlenhuth, E., *Roux's Arch. f. Entwicklungsmech.*, 1928, in press.

the animal had received only 3 injections. The height of the cells was increased, the number and size of the neutral red granules was larger than in the controls (and in normal standard animals in general) and the intensity of the neutral red stain was distinctly greater. This animal showed only a few signs of metamorphosis.

In another animal (CCXCIV b 3) the thyroid was examined unstained, after 3 injections had been given. The cells were distinctly enlarged and showed the typical dome-like protrusion of the apices, observed in active glands of spontaneously metamorphosing larvae. In this animal no definite start towards metamorphosis had been made.

In a third animal (CCXCIV b 1) which had received 5 injections, the cells exhibited characters indicative of the highest secretory activity. Some cells were found which were twice as high as the cells of control thyroids and contained one large Andersson vacuole at the apex. Other cells were merely increased in size; of these 50% contained so many Andersson vacuoles that little of the cytoplasma remained. This animal had shed its first skin completely one day before examination.

Our conclusion is that genuine anterior lobe extract has the same action on the thyroid as Armour's commercial product, that a fully potent extract can be obtained not only from dried, but from fresh glands; that the anterior lobe contains a substance which, alone among other substances tested and quite contrary to thyroid substance and substances stimulating the autonomic nerves, forces the inactive thyroid gland into function; and that, in amphibians, metamorphosis is caused by this principle only indirectly, through the function of the thyroid gland.

4191

Anterior Lobe Substance, the Thyroid Stimulator. IV. Effect in the Absence of Thyroid Gland.

S. SCHWARTZBACH and E. UHLENHUTH.

*From the University of Maryland Medical School.**

Spaul[1] reported that injections of anterior lobe substance induced metamorphosis in thyroidectomized axolotls. According to our work, however, the effects of the hormones are quite different and anterior lobe cannot replace the thyroid hormone.

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* These experiments were made possible through the generosity of Mr. Victor G. Bloede, Baltimore.

[1] Spaul, E. A., *Brit. J. Exp. Biol.*, 1925, ii, 427.

To test our claim 6 larvae of the Utah axolotle were most carefully thyroidectomized. As complete thyroidectomy of salamander larvae is very difficult and regeneration of remnants not visible under the dissecting microscope takes place frequently, the injections of an anterior lobe extract prepared in our laboratory, as described in Article I of this series, were started in 4 of the thyroidectomized larvae immediately (one day) after the operation. Two normal control larvae of the same stage and approximately same size were injected at the same time with the same sample of anterior lobe powder as the experimentals. Both controls metamorphosed after the sixth injection, 11 days after the first injection. None of the experimentals showed even a sign of metamorphosis after the ninth injection, fifteen days after the first injection. The injections were stopped after the ninth injection; 51 days after the first injection, the experimentals are still completely larval.

Anterior lobe substance has absolutely no effect on metamorphosis if the thyroid is absent. It is possible that Spaul's axolotles had not been completely thyroidectomized. Anterior lobe is a specific thyroid stimulator, but it cannot replace the thyroid hormone.

4192

Action of Several Disinfectants on Yeasts.

FRED W. TANNER AND GEORGE D. BOLLAS.

From the Department of Bacteriology, University of Illinois, Urbana.

Ten strains of yeasts were subjected to the action of gentian violet, mercurochrome-220, iodine and acriflavine. The technic involved the use of a plate very much like that used by Churchman. The plate which we used, however, had 2 control areas instead of one. The agar containing the dye was added to the middle compartment, leaving one compartment on each side without the disinfectant. Gentian violet was studied in 3 ways: (1) application of the dye directly to the cells; (2) behavior of the organisms on dye-agar; and (3) attempts to raise the tolerance of the organisms for the dye. All of the cultures except one were susceptible to the presence of gentian violet. This organism, when stained according to Henrici's[1] technic for applying the Gram stain to yeasts, was Gram negative. The observation that 9 of the yeasts were Gram positive explains their inability to endure the presence of gentian violet. It was found that when the dye was employed at a dilution of 1 to 80,000 no growth appeared on the dye media even after several days' incubation. On the other hand, a dilution of 1 to 140,000 allowed very slow growth after from 3 to 4 days incubation. Raising the temperature greatly increased the action of the dye. Attempts to raise the tolerance of yeasts to gentian violet were successful although the extent varied for each strain. These results indicate that yeasts, like bacteria, can be divided into two groups depending on their reaction to the Gram stain. Others who worked on this subject are E. B. Clark[2] and Faber and Clark.[3] Our results confirm their conclusions.

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[1] Henrici, *J. Med. Res.*, 1914, xxx, 409.

Mercurochrome 220 was studied in the same manner. It was less active on yeasts and required a much longer time to kill the fungi. A concentration of 0.4% repressed growth when the disinfectant was added to the medium. Mercurochrome-220 diffused quite rapidly from the dye agar into the plain agar so that no growth was secured near the line demarking the 2 kinds. No increased tolerance could be attained.

Tincture of iodine has had extensive use as a skin disinfectant. Consequently, a study of its toxic action on yeast cells was included in this investigation. Tincture of iodine was prepared by dissolving 5 gm. of potassium iodide in 5 cc. of distilled water. To this solution, 7 gm. of iodine were added and finally sufficient 95% ethyl alcohol to make 100 cc. This element was found to have a powerful action on yeasts. When tincture of iodine was incorporated in media in a concentration 1-100, the yeasts were completely destroyed. Attempts to increase the tolerance of yeasts to this element were entirely negative. Windisch[4] stated that the behavior of iodine with yeast could be classified as a special case of the Arndt-Schultz law. In very high dilutions the cell is stimulated by the iodine but in low dilutions the cells are killed.

The action of acriflavine on the strains used was quite variable. Five of the yeasts used were distinctly resistant. When incorporated in the medium, a dilution of 1 to 500 prevented growth.

Summary and Conclusions: The results may be briefly summarized as follows: Gram positive yeast, like bacteria, can be classified into gentian violet positive, while gram negative are likewise gentian violet negative. Yeasts which are gentian violet positive are destroyed when stained for one hour with 5 drops of an aqueous saturated solution of this dye. Yeasts which are gentian violet negative are unaffected by this treatment. When the dye is incorporated into media at a dilution 1 :80,000 it will completely destroy the fungi, while at a dilution of 1 :140,000 growth is inhibited. As the temperature increases the time required to destroy the stained organisms decreases, and increases as the temperature decreases. Tolerance towards gentian violet could be produced with most of the yeasts studied.

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[2] Clark, E. B., *J. Inf. Dis.*, 1926, xl, 423.

[3] Faber, H. K., and Clark, E. B., *Am. J. Dis. Child.*, 1927, xxxiv, 408.

[4] Windisch, W., *Woch. Brau.*, 1927, xliv, 516.

Mercurochrome-220 soluble when incorporated into media at a concentration of 0.4% destroys the yeasts planted on it. Yeasts treated with 5 drops of a 2% solution of mercurochrome are destroyed after 8 to 11 hours in perfect contact with the dye. No tolerance towards mercurochrome could be produced by the organisms studied.

Tincture of iodine, prepared according to U. S. Pharmacopoeia, will destroy yeasts instantaneously when one drop of this solution is applied to one cc. of a heavy suspension. At a dilution of 1 :50, yeasts are destroyed in less than 5 minutes when treated with only 2 drops of this dilution. When 5 drops are used it kills the organisms instantaneously. Tincture of iodine when incorporated into media in a dilution of 1 :100 will completely destroy the yeasts. No tolerance towards tincture of iodine could be produced with the organisms under study.

Neutral acriflavine has a marked bactericidal inhibitory action on some yeasts but in others its effect is practically insignificant.

Illinois Branch.

University of Illinois, October 30, 1928.

4193

Röle of Ectoparasites in Bartonella Infection of Albino Rats.

PAUL R. CANNON AND PRESTON H. McCLELLAND.

From the Department of Pathology and the Otho S. A. Sprague Memorial Institute, The University of Chicago.

The importance of the albino rat as an experimental animal makes *Bartonella* infections a serious problem in experimental medicine. In addition, the similarity of the *Bartonella* infection of rats to the human infection, Oroya Fever, suggests the possibility that the 2 diseases may have a number of points in common, particularly as to modes of transmission. This report deals with the latter problem.

Lauda[1] showed that the anemia resulting from splenectomy of albino rats was transmissible by injection of liver emulsion, although at that time he was unaware of the presence of *Bartonella* organisms in the infected animals. Later, Haam, Lauda and Sorge[2] discovered that uninfected rats might become infected after being kept in cages with animals that were known to be infected.

We have confirmed the results of Lauda as to the transmission of the infection by injection of liver emulsion from infected rats into normal ones, and have even transmitted the disease by the injection of less than one drop of blood from a *Bartonella* infected animal into a normal one. As normal controls we have used rats from the Wistar Institute strain. Thus far we have splenectomized 34 of these animals varying in age from 3 weeks to 15 months and have never observed any significant anemia. Furthermore, the spleen weight-body weight ratio of these has averaged approximately 0.25%, whereas the average in the case of over 75 *Bartonella*-infected animals is 0.75%.

The fact that uninfected rats may become infected by contact with infected ones suggested the possibility that ectoparasites might play an important rôle in this transmission. Three Wistar strain rats were placed in a small cage with a rat from the *Bartonella*-infected stock and known to be infested with lice and mites. Fifteen days later, all of the Wistar rats were splenectomized and all developed the typical anemia, fatal in one case. A control Wistar rat, isolated during this period and splenectomized at the same time had an essentially normal hemoglobin and blood picture during the entire period of observation.

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[1] Lauda, E., *Virchow's Arch. f. path. Anat.*, 1925, cclviii, 529.

[2] Haam, E., Lauda, E., and Sorge, G., *Klin. Wchnschr.*, 1927, vi, 2240.

Next, 12 lice *(Polyplax spinulosus)*, engorged with fresh blood, were picked from *Bartonella*-infected rats and placed on each of 6 Wistar rats, which were then kept in sterilized cages. At the same time, 2 Wistar rats from the same lot were isolated in sterilized cages. At the end of 15 to 19 days all of the animals were splenectomized. The 6 louse-infested Wistar rats developed the typical anemia with countless *Bartonella* organisms being present on the erythrocytes, whereas the 2 control Wistar rats showed no significant changes following splenectomy and no *Bartonella* organisms were seen in the blood smears.

Two rats known to be infected with the *Bartonella* were then carefully "deloused" by means of Tincture of Larkspur and kept in close contact with 5 Wistar rats for 11 days. The latter were then splenectomized and again placed in contact with the infected animals. No significant blood changes occurred in 30 days of observation in the case of 3 Wistar rats, and in 15 days of observation in the case of the other 2.

It is evident that the louse is an important vector of the *Bartonella* virus. In order to prevent spreading of the infection in rat colonies this ectoparasite must be eliminated. Furthermore, a similar mode of transmission may be in force in Oroya Fever. This disease has a regional distribution, is more prevalent among people living under poor economic conditions, and is believed by many of them to be spread by body lice. In any event, the rôle of an ectoparasite in conveying the *Bartonella muris* suggests that similar ectoparasites, particularly the body louse *(Pediculus corporis)* and the bed-bug *(Cimex lectularius)* may act as vectors of the *Bartonella* bacilliformis of Oroya Fever.

4194

Influence of Diarrhea upon Gastro-Intestinal Auto-Disinfection.

LLOYD ARNOLD AND THOS. G. HULL.

From the Department of Bacteriology and Preventive Medicine, University of Illinois, and Diagnostic Laboratory, State Department of Public Health, Chicago.

Young dogs (2 to 4 mos. old) were placed in warm and humid rooms (90° F. temperature, 85 to 90% humidity) for 3 days. They were given 100 cc. of cows' milk every 8 a. m., 12 noon, and 4 p. m. Half of the animals were given sanitary tap water and half were given polluted water (Chicago River water). No diarrhea developed in any of these 12 animals. Two hours before killing them in an ether chamber, they were given 100 cc. of their respective drinking water samples plus one agar slant of *B. prodigiosus*. The contents of various levels of the gastro-intestinal tract were diluted in broth and immediately seeded on agar plates. The relative distribution of *B. prodigiosus* was determined in relation to the concentration of the ingested suspension. Six puppies of about the same size were given the tap water and 6 the polluted water in ordinary tem-

CHART 1

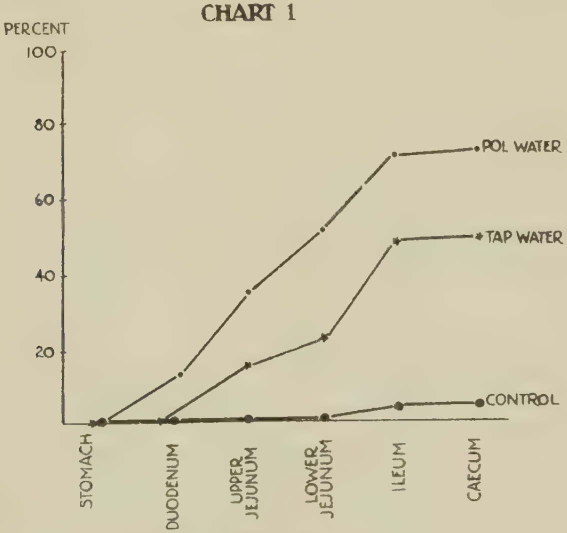

CHART I.

Plain tap and sewage polluted water.
Ordinate—% *prodigiosus* in relation to concentration fed by mouth.
Abscissa—segments of gastro-intestinal tract.
—o—o— Controls in ordinary temperature room, tap and polluted water the same.

perature rooms. Chart I shows the results. There was an interference with the intestinal bactericidal power in the warm room; this was most marked when polluted water was given instead of tap water. The same experiment was repeated, except that the water samples were brought to pH 8.0 with sodium carbonate solutions. The alkalinized polluted water caused diarrhea in 8 of the 12 puppies. The alkalinized tap water caused semifluid stools in 6 of the 12 puppies. Chart II shows the results. There was an inhibition in the bacterial killing power of the gastro-intestinal tract in the polluted water animals in this experiment.

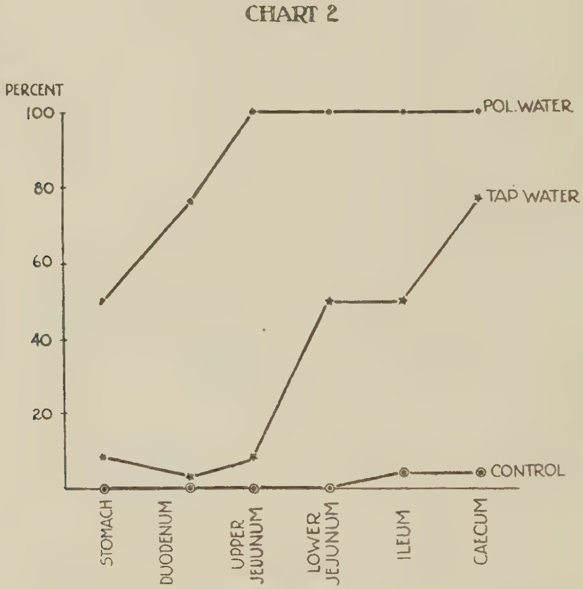

CHART II.

Alkalinized tap and sewage water.
Ordinate—% *prodigiosus* in relation to concentration fed by mouth.
Abscissa—segments of gastro-intestinal tract.
—o—o— Controls in ordinary temperature room, tap and polluted water the same.

Non-leaking gastric and appendicial fistulae were established upon 4 grown dogs. The small intestine was attached to the anterior abdominal wall at about the duodenojejunal junction. 300 cc. of cows' milk plus an agar slant of *B. prodigiosus* was ingested and specimens were removed at the same time from the stomach, small intestine and caecum. The acidity of the gastric contents was titrated for acid-deficit and free acid. The H-ion concentration of the intestinal contents was determined by colorimetric method. The

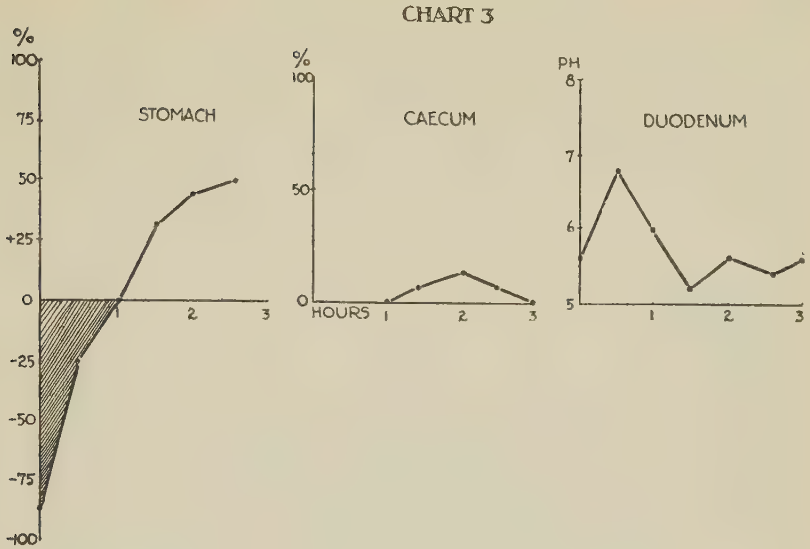

CHART III.
300 cc. plain milk plus *B. prodigiosus.*
Stomach—Ordinate—Acid deficit and free acid in clinical units.
Abscissa—Time in hours.
Caecum—Ordinate—% of ingested *B. prodigiosus* appearing in caecum in relation to concentration fed.
Abscissa—Time in hours.
Duodenum—Ordinate—pH of contents.
Abscissa—Time in hours.

caecal contents was diluted in broth and immediately seeded upon agar plates and the relative concentration of *B. prodigiosus* was determined in per cent as compared to the material fed to the animal. Chart III gives the results. The same experiment was repeated, except the milk was brought to pH 8.0 with sodium hydroxide. Chart IV gives the results. Young broth cultures of *B. shiga* were lysed with bacteriophage and the 150 cc. of the filtered material was diluted with an equal volume of broth and fed to these dogs plus a slant of *B. prodigiosus.* Specimens were removed and handled in the manner described for the previous experiment. Chart V gives the results. All 3 animals used for this experiment had diarrhea, *B. prodigiosus* was present in the fluid feces in large number.

The self-disinfecting power of the gastro-intestinal tract was inhibited in experimental diarrhea in young and adult dogs. Ingested bacteria are not destroyed in the usual manner. This would increase the hazard of infection if pathogenic bacteria were ingested during this period. There is a gastrointestinal irritant in polluted water. Water-borne out-breaks of diarrhea in humans fol-

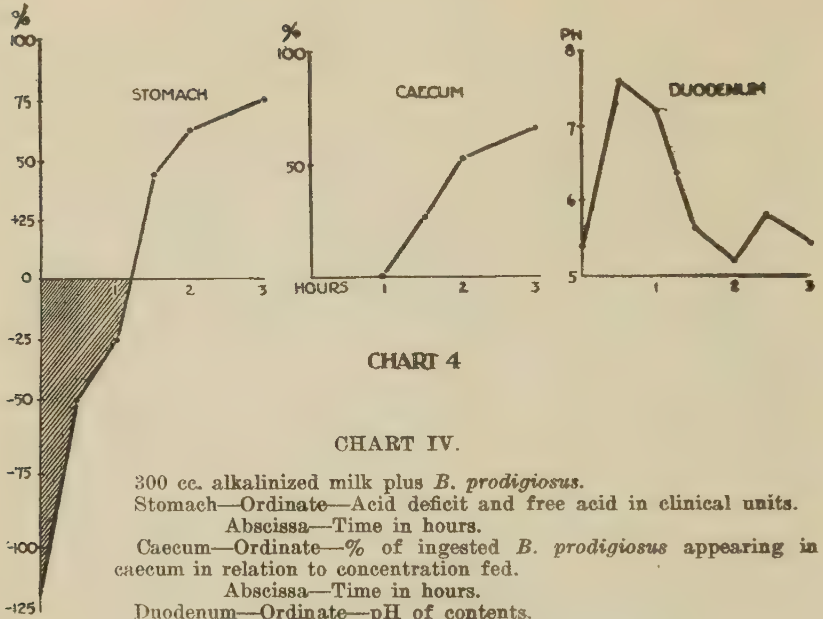

CHART 4

CHART IV.

300 cc. alkalinized milk plus *B. prodigiosus.*
Stomach—Ordinate—Acid deficit and free acid in clinical units.
Abscissa—Time in hours.
Caecum—Ordinate—% of ingested *B. prodigiosus* appearing in caecum in relation to concentration fed.
Abscissa—Time in hours.
Duodenum—Ordinate—pH of contents.
Abscissa—Time in hours.

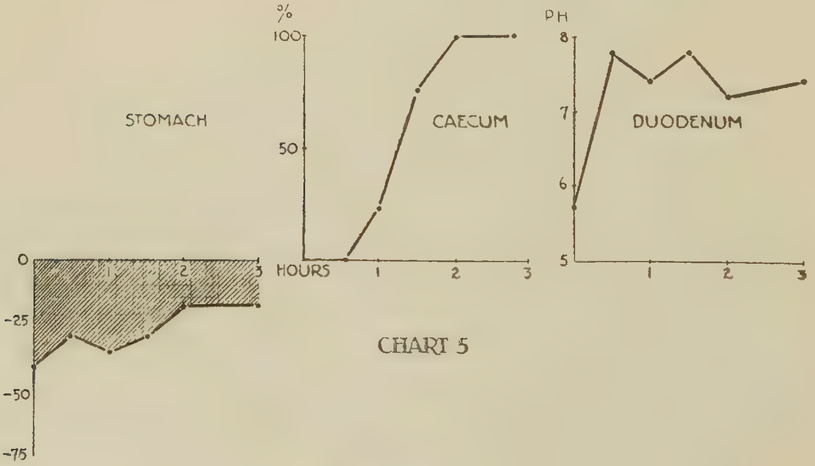

CHART 5

CHART V.

150 cc. *B. shiga* bacterial proteins.
Stomach—Ordinate—Acid deficit and free acid in clinical units.
Abscissa—Time in hours.
Caecum—Ordinate—% of ingested *B. prodigiosus* appearing in caecum in relation to concentration fed.
Abscissa—Time in hours.
Duodenum—Ordinate—pH of contents.
Abscissa—Time in hours.

lowing a pollution of the drinking water supply is well known. Chronic biliary typhoid carriers may be made large reservoirs of *B. typhosus* as a result of an attack of diarrhea. Epidemics of typhoid fever following a large water-borne diarrhea outbreak, may be due to disturbances in the equilibrium between parasites and host, namely, to increase in the distribution of *B. typhosus* and increase in the susceptibility of the host.

4195

Effect of Intratracheal Inoculation on Chronicity of Lung Abscess.

W. E. ADAMS, C. M. VAN ALLEN and LOIS A. DAY.

From the Department of Surgery, University of Chicago.

It has been amply demonstrated experimentally that the type of organism infecting a lung abscess is important in determining the healing time. Thus, when *Staphylococcus aureus* alone is introduced into the lung parenchyma of dogs, abscesses are produced that heal within 4 weeks[1]; when a mixture of the common pyogenic bacteria is used, the abscesses heal more slowly[2]; the spirillum and fusiform bacillus of Vincent give rise to abscesses that last 2 to 3 months[3]; and *Tubercle bacillus* abscesses may require a much longer time to heal.[4]

The purpose of the following experiments is to determine whether a lung abscess infected with an organism known to produce only acute lesions may be rendered slow to heal by intratracheal insufflation of organisms which are capable of originating chronic lung lesions. Dogs were used, and lung abscesses were obtained by liberating infected emboli into the venous stream (Cutler,[5] Holman[6]). Intratracheal insufflation was done with the animal deeply morphinized to control the cough reflex, and thinned sputum was used, obtained freshly from patients with chronic lung suppuration.

Group A. 14 dogs. Embolus with *Staphylococcus aureus*. 3

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[1] Van Allen, C. M., *Deutsche Z. f. Chir.*, 1928, ccix, 1.

[2] Holman, E., Chandler, L. R., and Cooley, C. L., *Surg., Gynec. and Obst.*, 1927, xliv, 328.

[3] Weidlein, I. F., and Herrmann, L. G., *J. Am. Med. Assn.*, 1928, xci, 850.

[4] Van Allen, C. M., Adams, W. E., and Day, Lois, unpublished work.

[5] Cutler, E. C., and Schlueter, S. A., *Ann. Surg.*, 1926, lxxxi, 256.

[6] Holman, E., Weidlein, I. F., and Schlueter, S. A., Proc. Soc. Exp. Biol. and Med., 1926, xxiii, 266.

died of pneumonia. 11 sacrificed at 30 days. Results: healed abscesses.

Group B. 6 dogs. Embolus with sputum from patient with bronchiectasis *(Fungus nocardia*[7]*)*. 1 died of pneumonia. 5 sacrificed at 30 days. Results: 4 with unhealed lung abscess cavities.

Group C. 6 dogs. Embolus with *Staphylococcus aureus.* 10 days later insufflation with sputum of bronchiectasis *(Fungus nocardia)*. 3 died of pneumonia. 3 autopsied at 30 days. Results: 1 with unhealed abscess cavity.

Group D. 9 dogs. Embolus with *Staphylococcus aureus.* 10 days later, insufflation with sputum of bronchiectasis (Vincent's organisms). 3 died of pneumonia. 5 autopsied at 30 days. Results: 5 with large unhealed abscess cavities.

Group E. 10 dogs. Embolus with *Staphylococcus aureus.* 10 days later, insufflation with sterile saline. 4 died of pneumonia. 6 autopsied at 30 days. Results: healed abscesses.

Group F. 21 dogs. Intratracheal insufflation, in 6 dogs of sputum of bronchiectasis, and in 15 dogs of sputum of bronchiectasis (organisms of Vincent). 9 died of pneumonia. 12 were autopsied at 10 to 30 days. Results: no abscesses.

It is to be seen that *Staphylococcus aureus* lung abscesses which heal regularly before 30 days, have frequently been made to persist as large, unhealed abscesses after 30 days by treating the animals on the 10th day with intratracheal insufflation of sputum infected with *Fungus nocardia* or Vincent's organisms. Insufflation with sterile saline does not have this effect. Insufflation alone with these organisms produces no abscesses.

We raise the question whether in man postoperative lung abscess which occurs from infected embolus may become superinoculated by mouth organisms through accidental insufflation and thereby assume the characteristics of bronchogenic abscesses.

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[7] Tunnicliff, R., and Huber, H. L., *Trans. Chicago Path. Soc.*, 1927.

4196

Influence of Various Ions on Fatigue Contracture.

H. H. DIXON* AND S. W. RANSON.

From the Northwestern University Medical School.

When 2 cc. of 1% sodium bicarbonate is injected intraperitoneally in frogs there results, in about one-half hour, a disorder of the skeletal muscles characterized by fascicular twitching and dystonic movements. This was described as tetany by Ringer[1] in 1884. A similar condition develops about one-half hour after the intraperitoneal injection of 2 cc. of a neutral 5% solution of sodium phosphates. We have not been able to produce this type of reaction by administration of sodium oxylate, sodium fluoride, potassium chloride, calcium chloride, magnesium chloride, ammonium chloride or hydrochloric acid, even when these substances were given in doses large enough to cause complete prostration.

We have studied the effect of all of these substances on the fatigue curve of the isolated gastrocnemius of the frog. The muscle was stimulated directly through silver chloride electrodes with faradic shocks delivered at the rate of 3 makes and breaks per second. Care was taken to have the stimuli always the same and strong enough so that the make shocks gave maximal contractions. The normal muscle when stimulated in this way responds at first with discrete contractions returning to the base line. After 15 or 20 seconds relaxation becomes delayed and fatigue contracture slowly develops resulting in a steadily maintained contracture of approximately the same height as the original discrete contraction.

A muscle from a frog treated with a neutral solution of sodium phosphates responds in a very different manner. Even after the first contraction, relaxation is much delayed and the muscle remains shortened with contractions of small magnitude superimposed upon the contracture curve. When the stimulation is discontinued, relaxation is slow and imperfect.

Strikingly different curves were obtained from the muscles of frogs poisoned with 0.4% sodium fluoride, the fluoride being substituted for that amount of sodium chloride in Ringer's fluid. When a frog was given 2 cc. of this solution prostration and muscular flaccidity occurred in about an hour and a half. Muscle isolated from such a frog responds in a normal manner for about a dozen contractions and then rapidly fatigues at the base line. This type of fatigue curve is due to the failure of contraction and is to be sharply distinguished from the fatigue contracture of normal and phosphate muscle, where the fatigue shows itself as a failure of relaxation. A point of special interest is that shortly after fatigue is complete the muscle begins to shorten. This shortening continues after cessation of the stimulus and the muscle rapidly goes into rigor. The lifting power of the fluoride muscle is not impaired at first as was shown by tracings made with 50 gm. loads.

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* Fellow of the National Research Council.

[1] Ringer, Sidney, *Brit. Med. J.*, 1884, ii, 114.

A 1% solution of sodium bicarbonate injected intraperitoneally into the frog alters the fatigue curve in much the same manner as phosphate. These observations are of interest in connection with the retention of phosphate in parathyroid tetany[2] and the development of tetany in normal individuals when alkalosis is produced by prolonged rapid and deep respirations.[3]

Sodium oxylate which is known to precipitate calcium in the blood stream[4] as well as *in vitro* and which might be expected to cause rigidity does not have this effect. Fatigue contracture develops just as slowly as in normal muscle. Calcium chloride, even when administered in quantities sufficient to prostrate the frog does not alter the fatigue curve from normal.

4197

Effect of Electric Currents on the Arteries.

R. H. JAFFÉ, D. WILLIS AND A. BACHEM.

From the Department of Pathology, University of Illinois.

In a series of experiments the effect of electric currents of low tension (110 to 220 volts) on the femoral artery of the dog has been tested. These currents do not cause any anatomical changes if the blood is circulating freely. Combination of the passage of the current with a stopping of the circulation at a point distal to the electrodes leads to a complete destruction of the muscle fibres of the media, while the elastic fibres retain their specific staining properties but lose their elasticity. It is suggested that the heat that is liberated in the vessel wall produces this effect. The circulating blood prevents the overheating of the wall. Hence, no changes result in the experiments in which there is no interference with the free circulation. Added proof to this explanation is the fact that a slight searing of the vessel with a hot wire leads to almost identical changes.

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[2] Herring, W., and Kuhnan, J., *Endocrinology*, 1927, xi, 80.

[3] Grant, S. B., *Arch. Int. Med.*, 1922, xxx, 355; *Am. J. Physiol.*, lxvi, 274.

[4] Vines, H. W. C., *Endocrinology*, 1927, xi, 290.

The vessels with the media practically gone become much dilated but they do not rupture. After several months the media has been replaced by a dense hyaline connective tissue with a scanty elastic network. No muscle fibres can be detected. The intima is thickened and also the adventitia has increased much in thickness and density.

4198

Comparison Between Morphine and Feeding as Agents in Production of Conditioned Salivary Response.

NATHANIEL KLEITMAN.

From the Department of Physiology, The University of Chicago.

In a quantitative study of the conditioned salivation in the dog developed through daily injections of morphine[1] it was found that the flow of saliva was unusually copious. Thus the total quantity of saliva secreted conditionally by a 12 to 14 kilo dog would run as high as 400 cc. in one hour. The conditioned stimulus in this case consisted in placing the dog in a stand where it remained for a definite period of time (usually 30 minutes), before receiving a subcutaneous injection of morphine. In the work of Pavlov and others[2] where food or acid was employed as an unconditioned stimulus, the "delay" between the beginning of the action of the conditioned stimulus and the application of the unconditioned stimulus was a matter of 2 to 3 minutes or even less. This suggested the possibility that the long "delay" employed in the case of the morphine-produced response might be responsible for the large amounts of saliva secreted. To test this, 3 dogs, with fistulae of the submaxillary glands, were placed in a stand for 30 minutes daily, for periods of 22, 27, and 76 days respectively. Each day, at the end of the 30 minutes in the stand, they were fed a meal of ground beef heart. The unconditioned salivary response to feeding was greater than to the injection of morphine (3-6 cc. compared to 1-3 cc.), but none of the 3 dogs showed any conditioned secretion before feeding. All these dogs developed the conditioned salivary response to morphine on subsequent occasions in the course of a few days.

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[1] Kleitman and Crisler, *Am. J. Physiol.*, 1927, lxxix, 571.

[2] Pavlov, "Conditioned Reflexes," 1927, Oxford University Press, New York.

It seems that in the development of conditioned salivation, using the same conditioned stimulus, daily injections of morphine are efficacious, whereas daily feeding is not.

4199

Spontaneous Extinction of Morphine Salivary Conditioned Response in Dog.

NATHANIEL KLEITMAN AND DOROTHY PALMER.

From the Department of Physiology, The University of Chicago.

The conditioned response to morphine is developed by placing a dog, with a fistula of the duct of the submaxillary gland, into a stand for a definite period of time daily, and injecting about 30 mgm. of morphine subcutaneously at the end of each period. The injection of morphine, among other effects, produces a flow of saliva. After a few days, placing the dog in the stand is found to act as a conditioned stimulus, salivation starting before the injection of morphine. If, while continuing the daily placing of the dog in the stand, the injections of morphine be stopped, the conditioned response becomes weaker and gradually disappears.[1] This has been termed experimental extinction, and, as had been shown by Pavlov and others, it always follows the repeated application of the conditioned stimulus without "reinforcement" by the unconditioned stimulus. The present investigation was undertaken to determine whether the conditioned response to morphine would persist when not experimentally extinguished. After the dogs had fully developed the conditioned response they were left alone for from 3 to 15 days, and at the end of that interval of time placed in the stand once more, and the magnitude of the conditioned response (if any) determined. In this manner we determined the percentage of retention of the response. Five dogs were used, and each was put through 2 or more "forgetting" tests. The following facts were established:

1. The conditioned salivary response to morphine becomes partially or completely extinguished spontaneously, within a few days.

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[1] Kleitman and Crisler, *Am. J. Physiol.*, 1927, lxxix, 571.

2. When the response is only partially extinguished, the percentage lost varies directly with the time elapsed.
3. Repeated establishment of the conditioned response leads to slower extinction.

4200

Lymph Production and Heat Regulation.

E. F. MÜLLER and W. F. PETERSEN.

From the Department of Pathology, University of Illinois, College of Medicine, Chicago.

Heat regulation in the mammalian body consists of a distinctly correlated reaction of almost all body organs toward changes of external temperature, among which the liver undoubtedly plays a most important rôle.

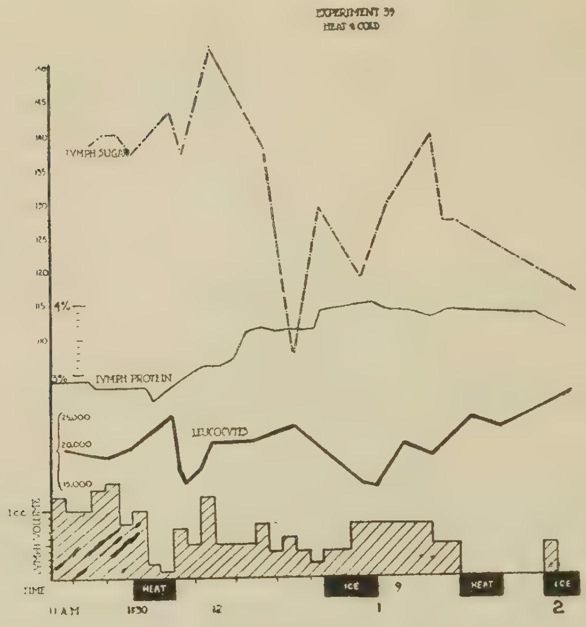
Changes of liver functions within this general reaction are only measurable from changes in the secretion from liver tissues. In order to draw some conclusions about the intensity of cellular activities, we have made experiments on lymph obtained from a thoracic duct cannula, since thoracic lymph originates almost entirely from the liver. It seemed advisable to test how cold or heat applied to the body surface of dogs would act upon lymph production in order to obtain further insight into the participation of the liver within the mechanism of heat regulation.

After a control period of about 1 hour (during which lymph was collected in 5 minutes samples) heat was applied by warm water bags or electric pads to the shaved abdominal skin while the dog was packed in cotton.

During such periods of heating a diminution could be observed, not only in lymph volume, but also a decrease of the percentage of lymph protein, indicating a possible decrease in cellular activity as well as in tissue permeability.

Application of cold (ice to the dog's skin by packing the animal into the ice mixture) altered the lymph production oppositely. Volume as well as the contents of lymph protein increased markedly under the influence of cold.

One of these tests charted below will best illustrate these correlations between the effect of temperature changes of the body surface and the alteration in lymph production as a manifestation of a simultaneously active liver reaction.



Usually we did not test heat and cold with the same animal, since it takes some time until the reaction toward the first temperature change cases.

Our observations show that intensive temperature changes applied to the body surface of dogs lead to an alteration in lymph production. This alteration must be the result of autonomic stimuli that reach the liver, since application of such cold or heat does not appreciably alter the temperature of deeper tissues. It has to be assumed that alteration of the body surface by marked temperature changes leads to a corresponding reaction of the peripheral tissues directly concerned which in turn gives rise to impulses that reach the liver, among other organs, and cause the described alteration of lymph production.

(1) Lymph production autonomically increases more or less markedly when intensive cold is applied to the body surface and decreases with the application of heat.

(2) This indicates that the autonomic reaction toward this alteration occurring in the peripheral tissues concerned (skin) is associated with a simultaneous transmission of impulses which stimulate, among other organs, the liver via preformed communications of the vegetative nervous system. The reaction produced is apparently oriented from that of the skin and other peripheral organs.

4201

Experimental Uremia. Uremic Enteritis.

M. H. STREICHER. (Introduced by E. Andrews.)

From the Department of Medicine, University of Illinois, and the Research and Educational Hospital, College of Medicine.

The objects of this research were to determine what effect uremia has on the Gastro-intestinal canal and to explain if possible the causation of uremia on a chemical basis.

Uremia was produced in dogs by repeated injections of 10% to 20% urea solution (sterile) intravenously, giving 200 cc. daily for 3 days at the rate of 5 cc. per minute (Woodyatt pump). One ureter had been ligated in many instances and in other cases the ureter had been intact. The blood, urine and feces were collected at 24 hour intervals for chemical determinations and at necropsy gross and microscopic tissue studies were made.

In about 40% of the dogs a picture similar to that of uremic coma, the so-called "Kussmaul picture", was produced after a single injection of 200 cc. of 10% or 20% urea solution and in practically 100% of the animals coma was invariably produced after the 2nd or 3rd injection.

The outstanding features of the chemical analysis may be grouped as follows: (a) A progressive acidoses demonstrated by a marked fall in CO_2 content. (b) A marked fall in blood proteins evident in the decrease of the refractometric index. (c) Marked rise in urine chlorides. (d) Disturbance in the calcium-potassium ratio—the ratio approaching 1 or 1+. (e) Marked increase in blood urea.

Upon careful analysis of the output of urea in the form of ammonia nitrogen and urea-nitrogen, it is evident that only 25% to 30% is recovered in the urine and feces.

A very interesting observation was made by Andrews that the identical chemical picture was produced by intravenous injection of a 5% sodium-chloride solution. He suggests that "The disturbance of the osmotic equilibrium resulting from maintaining high blood urea levels for a prolonged period causes or is associated with changes in the mineral salt balance which bears a striking resemblance of that found in human uremia or experimental uremia from sodium chloride injections."

Histologic examination showed the following changes: *Intestine*, marked enteritis; *Liver*, marked congestion—but no cellular changes; *Lung*, marked hyperemia; *Kidney*, marked hyperemia—

TABLE I. *Experimental Uremia.*
Chemical analysis of intravenous injection of urea solution—200 cc. of 20% given daily for 3 days. Rate of injection—5 cc. per minute. (Muriel H.)

	Before Inj.			72 hrs. after Inj.			Comment
	Urine	Bld.	Fec.	Urine	Bld.	Fec.	
Output	700 cc.		31G	650 cc.		68G	R. ureter ligated. Average wgt. of dog 17 kilos (female). Food given. Series of 15 dogs. Convulsions. Vomiting. Increased Resp. Diarrhea Coma. Death.
T. N.	4.7 G		3.9G	11.9G		1.4G	
NH_3N	0.4 G		0.02G	1.6G		0.34G	
Urea N	0.38 G	19 mg.	0.04G	7.1G	76mg	0.08G	
Albumin	—						
CO_2		52.4cc.			32.1cc.		
Chlorides		402mg.			492mg.		
Calcium		8.2 mg.			11.3mg		
Potassium		20.2mg			20.4mg		
Ref. Index		50			50		

Data for 24 and 48 hours after injection not recorded in table.

TABLE II. *Experimental Uremia.*
Chemical analysis of intravenous injection of urea solution—200 cc. of 20% given daily for 3 days. Rate of injection 5 cc. per minute. (Peggy O'C.)

	Before Injection			72 hrs. After Inj.			Comment
	Urine	Bld.	Feces	Urine	Bld.	Feces	
Output	750cc		22G	750cc		9G	Ureter not ligated. Average wgt. of dog 17 kilos (female). Food given. Series of 9 dogs. Convulsions. Vomiting. Cheyne-stokes breathing. Diarrhea. Coma. Death.
T. N.	4.1G		4.0G	16.5G		1.1G	
NH_3N	.3G		.03G	.6G		.1G	
Urea N	.4G	18mg	.05G	12G	148 mg	0G	
Albumin	—			+			
CO_2		50.8cc			32cc		
Chlorides		418mg			673mg		
Calcium		8.1mg			16.2mg		
Potassium		20mg			13.1mg		
Ref. Index		54			48		

cloudy swelling of the epithelium—obstruction of lumen of convoluted tubules.

Conclusions: Intravenous injection of 10% to 20% urea solution intravenously produces a marked enteritis. All clinical and chemical features of uremia may be reproduced even though both kidneys be intact.

4202

Experimental Edema.

LOUIS LEITER. (Introduced by P. R. Cannon.)

From the Lasker Foundation for Medical Research and the Department of Medicine of the University of Chicago.

The consistent occurrence of a low plasma protein level in patients with chronic renal edema led to the attempt to produce a similar state in experimental animals. In the acute experiments on dogs

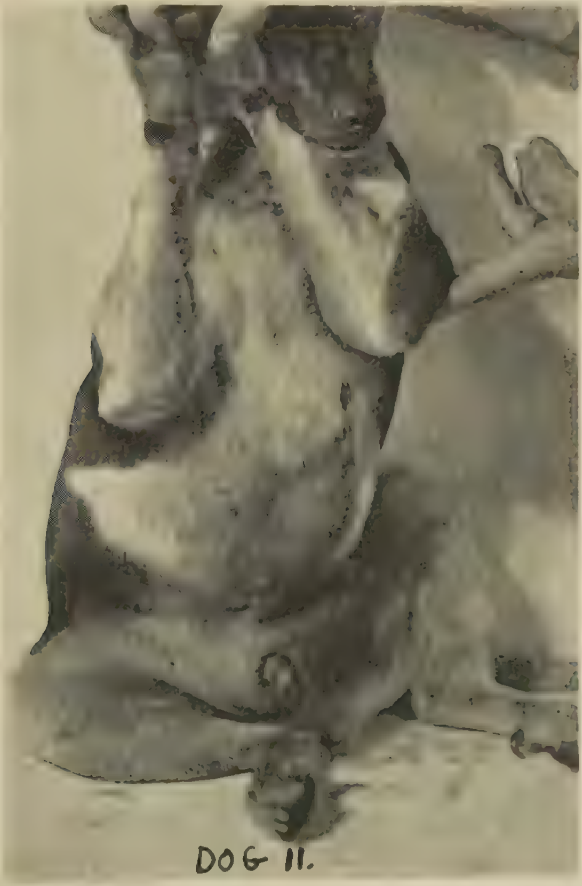


FIG. 1.

Dog 11. Male. Control weight 11.2 kg.; on the day of this photograph 12.8 kg. There is massive edema of the lower abdominal wall, external genitals, buttocks and thighs, less on the chest and upper abdominal wall. Constant seepage is occurring from needle-puncture wounds. Ascites was also demonstrated.

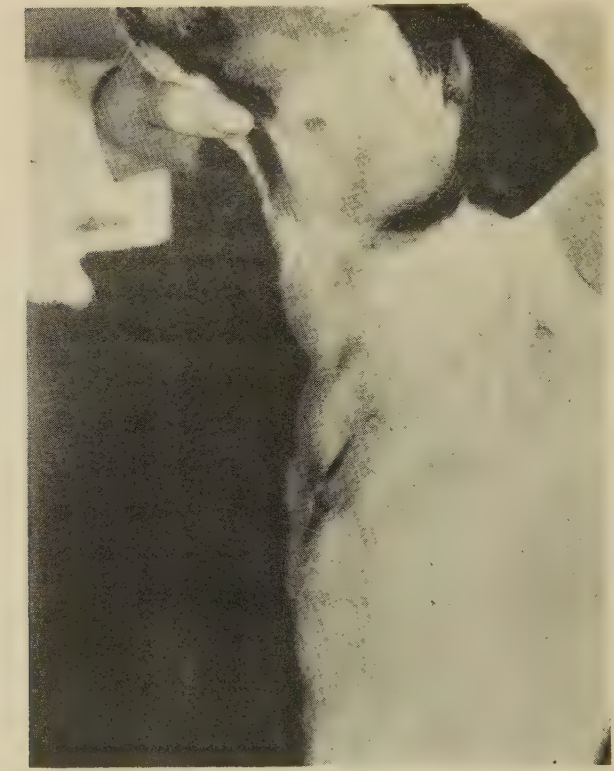


FIG. 2.

Dog 17. Male. Control weight 10.0 kg.; on the day of this photograph 12.8 **kg.** The rolls of edematous tissue on the neck are well shown. At the same time there were marked edema of the chest and abdominal walls and considerable ascites.

carried out several years ago in collaboration with J. F. McIntosh no edema was obtained except in 2 dogs and then not to any marked degree.

The present work is based on the development of a method for the production of *chronic* hypoproteinemia. Dogs are bled twice daily, 400 to 500 cc. of blood being withdrawn each time while the corresponding volume of erythrocytes suspended in modified Locke's solution is reinjected into the saphenous vein. 1500 cc. of 0.85% NaCl solution are given daily by stomach tube.

Edema usually appears about the fifth day, at first in the soft tissues of the prepubic region and the external genitals, later on the buttocks and thighs, and then on the abdominal and chest walls or even the neck. At the same time ascites develops, in association with hydrothorax and even pulmonary edema. The forelimbs are involved only in extreme cases. The weight of the dog increases sharply up to 30% or 40% above the control level in spite of decreased appetite and loss of flesh. The edema usually begins when the plasma proteins have fallen to 3% or less and recedes with a rise above that level if daily bleeding is discontinued. The ascitic fluid or subcutaneous edema fluid usually has a protein content of less than 0.25% and often below 0.1%. Most of the animals have died of accidents connected with the method used. Even massive edema and ascites, however, are compatible with good general condition of the dog.

Further work is being carried on to control the various factors involved in the production of this edema.

4203

Ultraviolet Radiation; Stimulation and Inhibition in Lower Organisms.

MARIE A. HINRICHS.

From the Department of Physiology, The University of Chicago.

The statement is sometimes made that radiation depends for its effectiveness as a stimulating or an inhibiting agent upon wavelength rather than upon dosage. Quality rather than quantity is the factor which decides whether an effect is to be stimulative or inhibitory. However, a number of workers, among them Bovie, L. Loeb, and Packard, using ultraviolet, x-ray, and radium, respectively, found that it is possible to produce both types of effect with the same region of the spectrum. A survey of about 5 years' work with the lower animals shows this to be the case in at least 2 regions of the spectrum, ultraviolet, and the visible region (following the action of sensitizing dyes). While the experiments were originally not conducted to test this point, it soon became apparent that with the same spectral region, short exposures produced primarily stimulation, and longer exposures, depression. Later work included experiments in which the rate of a physiological process was modified in either of 2 directions following exposure for short and long periods to the unscreened radiation from a quartz mercury-vapor arc.

Such studies were made with yeast. Ordinary baking yeast was suspended in distilled water and exposed, in open dishes, to radiation from the quartz mercury-vapor arc at various distances from the center of the arc. In these experiments, distilled water was used because it had previously been found that the action of ultraviolet radiation upon the constituents of the ordinary nutrient solution produces substances which are toxic to yeast. Earlier studies made by Woodrow, Bailey, and Fulmer reported only depression of sugar-fermenting power as a consequence of the action of ultraviolet radiation upon the nutrient solution itself.

In our experiments it was found that the sugar-fermenting power of yeast, as measured by rate and total amount of carbon dioxide production for a given period, could be stimulated or inhibited. The results may be summed up as follows:

1. 75% of all cases which produced stimulation were the results of exposures of less than 10 sec. in duration, at from 20 to 73 cm. distance from the center of the arc.
2. 97% of the cases which produced depression at from 20 cm. to 56 cm. were due to exposure of 30 sec. or longer.
3. The effect of radiation, whether it be stimulative or inhibitory, is transitory, and the rate of carbon dioxide production returns toward normal after an interval of several hours or longer, depending on the degree of stimulation or inhibition originally produced. In some cases, the effect was permanently inhibitory, and no recovery occurred. Where the effect was temporary, a gradual return to normal was found to be followed by a slight advance beyond normal in the other direction. For example, slight inhibitions of rate of fermentation may gradually disappear, and an actual increase in rate may follow.
4. Preliminary studies indicate that the rate of cell division is affected by radiation, which probably largely accounts for the increase or decrease in the rate of carbon dioxide production of a given mass of yeast suspension, although the effect on metabolic activity and enzyme production of the single cell, as well as the effect of radiation on the rate of enzyme activity, cannot be excluded.

In our experiments to date, short exposures to radiation stimulate, and longer exposures depress. This was found to be true whether the studies were being made upon cell division, as in the case of Paramecium, on embryological development, or on physiological processes such as the activation of starfish eggs or the stimulation of frog gastrocnemei to contraction. Developmental modifications may proceed in either of 2 directions, *i. e.*, they may appear as differential inhibitions or differential stimulations, as in the case of *Arbacia punctulata*. The more slowly developing vertebrate embryos are difficult to stimulate differentially because the short period of radiation loses its effectiveness before it can register a modification of developmental processes. The longer exposures are, however, certain to produce differential inhibitions. By this is meant that the regions of highest metabolic activity, at the time of exposure to radiation show the greatest inhibition in later development. In the salt water minnow, *Fundulus heteroclitus,* we find modifications in the brain and head region, the eyes, and the circulatory system, particularly the heart, when exposures are made during early stages of development. Exposures made before the 2-cell stage are often the cause of production of 2-headed forms. When exposures are made later in development (when the tail region is developing) malformations of the tail are produced.

Similar studies were made upon the chick with comparable results. In these experiments, windows were sawed through the shell of the egg, and exposures were made of the developing blastoderm at various stages, and then the shell was again closed and sealed and the egg returned to the incubator to complete its development to the desired stage. Cross-sections of 72-hour embryos show that radiation causes differentiation of brain cells to be interfered with, while proliferation continues. The brain of such a chick is a large mass of irregular folds which cannot be interpreted as normal convolutions when compared with the control animals of the same stage of development.

Differential stimulation or inhibition in the development of *Arbacia,* the increase or decrease in the production of fertilizin by *Arbacia* eggs, and the corresponding increase or decrease in fertilizability of such eggs, the increase or decrease in the rate of division of Paramecium, the shortening or lengthening of the period of division in the same form, are examples of the effect of short and long exposures to radiation, respectively. In Paramecium it was possible so completely to inhibit division in a cell which was about to divide, that the process was never fully accomplished and the pair of cells was permanently united. Successive exposures at the time of division of the members of such a pair again inhibited division, and permanent chains of 3 and 4-celled animals resulted.

From the experiments with yeast, and the other experiments described above, it seems reasonable to conclude that the factor which determines whether the effect of radiation shall be stimulative or inhibitory, is a quantitative rather than a qualitative one. In other words, the amount of radiation in a given region of the spectrum, which is absorbed by the protoplasm in a given period of time, determines whether there is to be an increase or decrease in the speed of its physiological processes.

4204

Factors Affecting Arterial Supply of Testis Under Experimental Conditions.

Y. JORANSON, V. E. EMMEL and H. J. PILKA.

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The presence of well defined anastomoses between the 3 arteries of the testis at the *cauda epididymidis* has been repeatedly demonstrated by morphological studies. The testicular atrophy, however, that has followed experimental and clinical ligation of the internal spermatic vessels, indicates the need of an investigation of factors affecting the arterial supply of the testis. It has been shown that after ligation of the internal spermatic artery and vein, the testis undergoes sloughing or complete atrophy while the epididymis remains normal. This difference toward ligation became a primary consideration in the present investigation. The *tunica albuginea* covering the epididymis is thinner and less resistant than the part covering the testis. Was the congestion in the testis following ligation due to the unyielding nature of its fibrous investment?

A characteristic reaction associated with ligation of the internal spermatic vessels is a sudden appearance of firmness or so-called "stone-hardness" in the testis, and this stone-hardness is remarkably uniform in degree. Our objective was to duplicate this increased tension experimentally and test the degree of penetration of injection-media under these conditions. Hill, while injecting the human testis, observed that the organ became firm when perfused with normal salt solution under a pressure of 60-80 mm. of mercury. This increase in tension occurred with a pressure below the normal systolic pressure, and Hill believed that the phenomenon was due to an extravasation of fluid between the endothelial cells of the capillaries, induced by the rapid death of these cells.

In the present work we have been able to duplicate the "stone-hardness" of the testis by means of saline perfusions. A control condition which will here be designated as "normal tension" was maintained with saline perfusions of a duration of 4 minutes, under a pressure not exceeding 120 mm. of murcury. A degree of tension, which will here be used as the standard of "increased tension", was produced by saline perfusions under the same pressure prolonged to 20 minutes. This increased tension corresponds to the stone-hardness found in the living testis after ligation of the internal spermatic vessels.

The degree of penetration of separate injections of 1.25% solution of celloidin in acetone and 2% solution of Berlin blue in distilled water, was determined under normal and increased tension of the testis. The perfusion and injection were made into the segment of the aorta corresponding to the origin of the internal spermatic artery and during the procedure the whole animal was submerged in water. Arterial casts were made by placing the celloidin specimens in 75% hydrochloric acid for 24 hours.

In the first series of 8 dogs in which normal tension was maintained in the testis, the celloidin extended into the finest radicals of the internal spermatic artery within the testis and epididymis, passing through the arterial anastomoses into the deferential and external spermatic arteries. An extensive anastomosis was found between the 3 arteries in the spermatic cord.

In the second series of 8 dogs, in which the standard of increased tension was produced, the celloidin penetrated only the larger arterial rami of the testis, and failed to pass beyond the *tunica albuginea* into the septa in the interior of the organ; the penetration of the epididymis in several specimens was quite complete, reaching and passing beyond the spermatico-deferential and spermatico-cremasteric anastomoses.

The above results were confirmed in a third series of 4 dogs, in which the penetration of a less viscid fluid, Berlin blue in distilled water, was determined, 2 under normal and 2 under increased tension. The findings were similar to those obtained in the preceding series. Again under normal tension both testis and epididymis were equally well injected. On the other hand, under increased tension the difference in penetration of the dye in the testis and epididymis was even more striking than that of the celloidin. While the epididymis was again completely injected, the testis was penetrated to the extent of only 1 mm. from the periphery.

The present results demonstrate that definite pressure disturbances upon the vascular system of the testis occur after prolonged saline perfusions. Comparable disturbances may occur in the testis after ligation of the internal spermatic vessels. In our experimental injections this disturbance was measured by the penetration of the celloidin or Berlin blue; in experimental ligation it is determined by the degree of atrophy. The extent to which the degree of penetration in experimental injections is equivalent to the degree of atrophy following ligation, remains a problem for further investigation.

The difference in the behavior of the testis and the epididymis toward injections under increased tension and toward ligation of the internal spermatic vessels appears to be due to the fact that the portion of the *tunica albuginea* overlying the testis is thicker and more unyielding than that covering the epididymis. If pressure within the capsule after ligation is a factor in the production of atrophy in the testis, then either an anterior longitudinal incision through the *tunica albuginea* or a division of the testis in the mid-line with a subsequent repair by suture of the *tunica vaginalis,* may relieve the pressure and thus prevent atrophy. The results of a series of experiments now being conducted with a view to testing this hypothesis will be given in a subsequent report.